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# THE
# ANATOMICAL RECORD

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# PROGRAM AND ABSTRACTS FOR THE TWENTY-SIXTH ANNUAL MEETING OF THE AMERICAN SOCIETY OF ZOÖLOGISTS

NEW YORK CITY, DECEMBER 27–29, 1928

These titles and abstracts make up the 1928 program of the American Society of Zoölogists. They were received on or before November 15th either by the Secretary of the Society, or by the Secretary of the Joint Genetics Section of the Society and the Botanical Society of America, and have been arranged according to the accepted program rules.

The abstracts are in the form contributed by their authors. They have been edited only to secure a uniform system of titles and style and to reduce length when obviously overlong. Their arrangement and the planning of the program can be done only after the titles are all at hand and must be greatly rushed in order to obtain publication before the meetings. The Wistar Institute is scheduled to mail out this issue of The Anatomical Record three weeks, to the day, after receiving the manuscript. All proofreading will be done there. The Institute deserves great credit for making possible the early publication and distribution of this material.

These abstracts and the accompanying data are presented as a part of the official proceedings of the 1928 meetings of the Society. The minutes of these meetings and a list of the officers of the Society for 1929 will appear in the January number of this journal.

THE ANATOMICAL RECORD, VOL. 41, NO. 1
DECEMBER, 1928

## THE AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE

The registration fee for the New York meeting is to be one dollar, payable on arrival at the registration offices of the Association, University Hall, Columbia University. This fee will be remitted to life members of the Association, to annual Association members who have paid their annual dues for the current year, and to associates for the meeting. Members who bring their blue receipt cards will avoid a slight delay, but the Association records will be at hand and each person's record can be promptly verified. If dues have not previously been paid, they may be paid at the Registration Offices, and the dues of new members will also be received there. New members pay the regular five-dollar entrance fee and the first annual dues (five dollars) and their membership subscription to the journal (''Science'' or the ''Scientific Monthly'') begins January 1st. But the entrance fee is now remitted in the case of members of any organization officially affiliated with the Association. Associates for an annual meeting pay the associate fee of five dollars and do not receive the journal.

Registration entitles the person registering to a copy of the General Program and its supplements, to the official badge, to all the general privileges of the meeting, and to the validation of one railway certificate. Also, every person registering is to receive, about February 1st, the special issue, or issues, of ''Science'' that contain the general reports of the meeting. Members of the Association who take ''Science'' regularly will automatically get this special issue, which will also be sent, by a special mailing, to those who regularly take the ''Scientific Monthly'' and to all non-members who register at the New York meeting.

The registration fees paid will be used to help defray the costs of the meeting.

Every person who travels by railway to attend the meeting should be sure to ask for and secure from the railway ticket agent, at the time he purchases his ticket to New York, a certificate ''for the New York convention of the American Association for the Advancement of Science and Associated Societies.'' The name of the Society need not be mentioned specifically. These certificates are to be presented at the registration offices of the A. A. A. S. upon first arrival at the meetings, where they will be indorsed and validated. After validation at New York, the certificate will entitle the holder to a half-fare rate for the return trip, for which he will purchase a ticket at New York, presenting the validated certificate to the New York ticket agent. Those arrangements for reduced railway rates are to be available for practically the whole of the United States and Canada.

# DIRECTIONS FOR REACHING THE McALPIN HOTEL (HEADQUARTERS HOTEL) AND COLUMBIA UNIVERSITY

The McAlpin Hotel is located at Broadway and 34th Street. Members arriving at the Pennsylvania Station will find the McAlpin Hotel one block east on 34th Street. From the Grand Central terminal it will be most convenient to take a taxicab. Baltimore and Ohio trains connect with buses (no extra fare), which one can leave at the Waldorf Hotel—a short walk from the McAlpin. Members arriving on the Delaware, Lackawanna and Western or the Erie, take Hudson tubes to 33rd Street—one block from the McAlpin. From the Jersey Central Station, take ferry to 23rd Street and a taxicab for the remainder of the distance.

Registration and the opening meetings of the Society will be at Columbia University, 116th Street and Broadway. To get to Columbia from the Hotel McAlpin (Headquarters Hotel) take the Seventh Avenue (not the B. M. T.) Subway (station one block west on 34th Street). Board any up-town train on the Broadway branch. Such trains carry on the front car (above) two red, a red and a white, or two white lights; never a green light. Avoid trains marked Lenox Avenue or Bronx Park. Express trains will save time.

Get off at the Columbia University Station, 116th Street. Walk east (away from the River) on 116th Street, pass up the stairs in front of the Library and around the Library to University Hall (an unfinished building with two large iron smokestacks). Register in University Hall.

Leaving University Hall, turn either to right or left, descend the stairs and cross to Teachers College, which occupies the block directly in the rear of University Hall. Floor-plan diagrams and other information will be available in the entrance halls.

# PROGRAM[1]

## THURSDAY, DECEMBER 27TH

10.00 A.M. *a.* Comparative and General Physiology (Animal Reactions and Sensory Physiology). Papers 1 to 12, inclusive. Macy Hall, Room 330.
  *b.* Protozoölogy, Comparative Anatomy, and Miscellaneous (concluded). Papers 78 to 81, 91 to 94, 108 to 109, inclusive. Main Hall, Room 300.
  *c.* Joint Genetics Sections. Dodge Hall, Room 304.

1.00 P.M. Buffet Luncheon given by the New York Zoölogical Society at the New York Zoölogical Park.

2.00 P.M. *a.* Demonstrations and exhibits. Dodge Hall, Rooms 301 and 302.
  *b.* Joint session of Genetics Sections with Geneticists Interested in Agriculture. Symposium: ''Inheritance of disease resistance in plants and animals.'' Leaders: H. K. Hayes and L. J. Cole. Main Hall, Room 301.

3.00 P.M. Motion-picture demonstrations. Program to be announced. Main Hall, Room 400.

4.00 P.M. Demonstrations and exhibits of Genetics Sections. Dodge Hall, Room 305.

4.30 P.M. Motion Picture—''The story of Antivenin.'' R. H. Hutchison and R. E. Stadelman. Horace Mann Auditorium.

8.30 P.M. Opening session of A. A. A. S. Address by Prof. Charles P. Berkey, followed by a general reception. American Museum of Natural History.

9.30 P.M. Zoölogists' Smoker. American Museum of Natural History, School Service Building.

## FRIDAY, DECEMBER 28TH

9.00 A.M. *a.* Cytology and Embryology (concluded). Papers 115 to 121, 135 to 145, inclusive. Macy Hall, Room 330.
  *b.* Joint session with the American Society of Parasitologists. Papers 158 to 159 will be read in conjunction with their program. Dodge Hall, Room 103.
  *c.* Joint Genetics Sections. Dodge Hall, Room 304.

12.15 P.M. Business session of Genetics Sections. Dodge Hall, Room 304.

1.30 P.M. *a.* Comparative and General Physiology (Endocrinology). Papers 13 to 21, inclusive. Macy Hall, Room 330.
  *b.* Demonstration papers of Joint Genetics Sections. Dodge Hall, Rooms 304 and 305.

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[1] All buildings mentioned in the program are a part of Teachers College, Columbia University, unless otherwise listed.

3.30 P.M. Business session of Section F. Macy Hall, Room 330.

3.40 P.M. Business session of the American Society of Zoölogists. Macy Hall, Room 330.

4.30 P.M. Motion Picture: Canti film of living cells in vitro. Dr. C. A. Kofoid. Main Hall, Room 400.

7.00 P.M. Zoölogists' Dinner, McAlpin Hotel (Headquarters Hotel). Address by Dr. C. E. McClung, retiring Vice-President for Section F, on "The nature of biological papers and the obligations of their authors." All biologists are cordially invited.

### SATURDAY, DECEMBER 29TH

9.00 A.M. *a.* Comparative and General Physiology (Biochemical subjects). Papers 22 to 39, inclusive. Macy Hall, Room 330.

*b.* Joint session with the Ecological Society of America. Papers 163 to 167 will be read. The remainder of the program will consist of papers of general interest to Zoölogists, presented by members of the Ecological Society. Main Hall, Room 301.

*c.* Joint Genetics Sections. Dodge Hall, Room 304.

2.00 P.M. Symposium under the auspices of the American Society of Naturalists. Subject: "The neuromuscular system." Speakers: G. N. Calkins, C. V. Taylor, Wm. Seifriz, and G. H. Parker. American Museum of Natural History.

Following the symposium, a motion picture of wild life, provided by the American Museum of Natural History, will be presented under the auspices of the local committee of Section F, Dr. G. K. Noble, Chairman. Main Auditorium, American Museum.

7.00 P.M. Dinner of the American Society of Naturalists.

# LIST OF TITLES

## COMPARATIVE AND GENERAL PHYSIOLOGY

### *Read*

1. Experimental study of spawning reactions of the female and male oysters. Paul S. Galtsoff, U. S. Bureau of Fisheries. (Lantern; 10 min.)
2. Periodic reversal of heart beat in Lepidoptera. John H. Gerould, Dartmouth College. (Lantern; 15 min.)
3. X-ray effects in relation to physiological condition in Paramoecium. Robert T. Hance, University of Pittsburgh. (Lantern; 5 min.)
4. The ionization and voltage of x-rays in relation to biological reactions. Robert T. Hance, University of Pittsburgh. (Lantern; 5 min.)
5. Behavior of amebas in a magnetic field. A. A. Schaeffer, University of Kansas. (Lantern; 15 min.)
6. The persistence of an instinct in Microstomum caudatum. Wm. A. Kepner and J. W. Nuttycombe, University of Virginia. (Lantern; 10 min.)
7. The effect of light on the eyes, pigmentation, and behavior of the cave salamander, Typhlotriton. G. K. Noble and Sarah H. Pope, American Museum of Natural History. (Lantern; 15 min.)
8. Further notes on color changes induced in fishes by light rays of varying wave length. Ellinore H. Behre, Louisiana State University. (10 min.)
9. The rate of dark adaptation in the eye of Eristalis tenax—a preliminary report. William L. Dolley, Jr., University of Buffalo. (Lantern; 15 min.)
10. Chroma-vision in the honeybee. L. M. Bertholf, Western Maryland College. (Introduced by S. O. Mast.) (Lantern; 15 min.)
11. The effect of dislocation of the eye and of unilateral blinding on the orientation and equilibrium in fish. Theodore Koppányi, Syracuse University. (15 min.)
12. The olfactory organs in the meal-worm beetle, Tenebrio molitor. J. M. Valentine, Western Reserve University. (Introduced by J. Paul Visscher.) (10 min.)
13. The effect of the injection of thyroid extract on the polynuclear count in a perennibranchiate amphibian (Necturus maculosus). Harry A. Charipper, New York University. (Introduced by Alden B. Dawson.) (Lantern; 10 min.)
14. The effect of thyroxin injection on the hair color, hair growth, and thyroid-gland structure in Peromyscus. Ralph R. Huestis and Harry B. Yocom, University of Oregon. (Lantern; 10 min.)
15. Comparative metamorphogenic properties of three iodized isomeres of tyrosin (di-iodo-paratyrosin, di-iodo-metatyrosin, and di-iodo-orthotyrosin) on R. temporaria larvae. Alexander B. Gutman, Cornell University and University of Halle. (Introduced by H. D. Reed.) (12 min.)
16. The biological significance of amphibian parathyroids. Roy A. Waggener, Cornell University. (Introduced by the Secretary of Section F.) (12 min.)
17. Hypophysectomy in the brown Leghorn fowl. J. B. Mitchell, Jr., University of Georgia. (Introduced by F. R. Lillie.) (Lantern; 10 min.)

18. Effect of anterior-lobe substance on basal metabolism in salamander larvae. Saul Schwartzbach and Eduard Uhlenhuth, University of Maryland Medical School.
19. The effects of subcutaneous injections of extracts of wolffian body on the growth and sexual development in the albino rat. C. D. Day, Kansas University. (Introduced by H. H. Lane.) (15 min.)
20. The production of hypertrophy of the müllerian ducts in castrated males of Triturus viridescens by ovarian grafts. A. Elizabeth Adams, Mount Holyoke College. (Lantern; 15 min.)
21. The effects of early ovariotomy in the brown Leghorn fowl. L. V. Domm, University of Chicago. (Lantern; 15 min.)
22. Antibody formation against an animal's own tissue. Paul S. Henshaw, University of Wisconsin. (Introduced by L. E. Noland.) (10 min.)
23. Some effects of crowding, pollution, food, and other factors on growth of the pond snail, Lymnaea stagnalis appressa. Edward D. Crabb, University of Pennsylvania. (Lantern; 10 min.)
24. The nutritional requirements of brook trout and their rate of growth. C. M. McCay, W. E. Dilley, and M. F. Crowell, Cornell University and Connecticut State Hatchery. (Introduced by the Secretary of Section F.) (Lantern.)
25. The effect of lethal concentrations of salts on fish. R. T. Young, University of Montana. (Lantern; 15 min.)
26. The effects of divalent and monovalent cations on locomotion in Amoeba proteus. Dwight L. Hopkins, Duke University. (Lantern; 15 min.)
27. Fertilization of marine ova in dilute sea-water and its significance for some problems in cell physiology. E. E. Just, Howard University. (Lantern; 15 min.)
28. Anaesthesia and cell division in sea-urchin eggs. Reuben Blumenthal, Marine Biological Laboratory and University of Pennsylvania. (Introduced by J. H. Bodine.) (Lantern; 5 min.)
29. Specific effects of low temperature on marine egg cells. Nellie M. Payne, University of Pennsylvania. (Introduced by J. H. Bodine.) (Lantern; 5 min.)
30. The effects of acids and alkalies on the viscosity of Arbacia-egg protoplasm. L. G. Barth, University of Chicago. (Introduced by L. V. Heilbrunn.) (Lantern; 15 min.)
31. The rate of swelling of Arbacia eggs in solutions of ammonium salts. Dorothy R. Stewart, University of Pennsylvania Medical School. (Introduced by M. H. Jacobs.) (7 min.)
32. Potential differences across the chorion of the Fundulus egg. Margaret Sumwalt, University of Pennsylvania Medical School. (Introduced by M. H. Jacobs.) (8 min.)
33. The influence of oxygen tension on the oxygen consumption of marine fishes. F. G. Hall, Duke University. (Lantern; 12 min.)
34. The effect of temperature on the rates of oxygen consumption in insects. Edith Rogers, University of Pennsylvania. (Introduced by J. H. Bodine.) (Lantern; 5 min.)
35. Effect of acidosis in strychnine poisoning. W. F. Wenner and E. W. Blanchard, State University of Iowa. (Introduced by W. W. Swingle.) (5 min.)

36. Effect of intravenous injections of alkali on the physiological action of curare. W. F. Wenner and E. W. Blanchard, State University of Iowa. (Introduced by W. W. Swingle.) (5 min.)
37. The effect of changes in hydrogen-ion concentration of the blood upon reflex time. W. F. Wenner and A. B. Taylor, State University of Iowa. (Introduced by W. W. Swingle.) (5 min.)
38. A study of the rate of oxygen consumption of different Cladocera clones derived from a single mother. V. Obreshkove and A. M. Banta, Saint Stephen's College, Columbia University, and the Department of Genetics of the Carnegie Institution of Washington. (15 min.)
39. Suggestions as to the true nature of 'respiratory X.' Edwin B. Powers, University of Tennessee. (Lantern; 15 min.)

### *By demonstration*

40. Apparent relation between peristomal gland cells and endodermal secretory cells of Palmatohydra oligactis (Pallas). C. H. McConnell, University of Virginia. (Introduced by Wm. A. Kepner.)
41. Charts illustrating the effect of light on the metamorphosis of the larvae of ascidians. Caswell Grave, Washington University.
42. The body size and the rate of fission in individuals of the protozoan Colpoda. Edward F. Adolph, University of Rochester.
43. The effects of gonadectomy in Triturus viridescens. A. Elizabeth Adams and Elizabeth S. Kirkwood, Mount Holyoke College.
44. The effects of ovariotomy in the brown Leghorn fowl. L. V. Domm, University of Chicago.
45. Demonstration of the positive action of the placental hormone on the oviduct of fowls. Mary Juhn, University of Chicago, and R. G. Gustavson, University of Denver. (Introduced by F. R. Lillie.)
46. A rapid quantitative precipitin technique. Alan Boyden and Joseph G. Baier, Jr., Rutgers University. (Introduced by Thurlow C. Nelson.)

46 a. The effect of $CO_2$ on the respiration of the duck. Theodore Koppányi, Syracuse University.

### *By title*

47. The pedal rhythm of the rock barnacle. William H. Cole, Rutgers University.
48. Behavior and reactions of a larval trematode, especially to changes of light intensity. Harry M. Miller, Jr., Washington University.
49. Relation between rate of locomotion and intensity in the beetle Dineutes americanus. Leonard B. Clark, Johns Hopkins University. (Introduced by S. O. Mast.)
50. Artificial insemination of female guinea-pigs as a method of investigating problems in the physiology of mammalian spermatozoa. William C. Young, University of Chicago and Brown University. (Introduced by Carl R. Moore.)
51. Methods of inducing the production and the hatching of sexual eggs of Daphnia longispina. Thelma R. Wood, Carnegie Institution of Washington. (Introduced by A. M. Banta.)
52. Regeneration in Daphnia magna. B. G. Anderson and L. A. Brown, State University of Iowa. (Introduced by W. W. Swingle.)

53. Histolytic influence of atrophying gills of anurans during metamorphosis, with special reference to resistance of fore-limb integument. E. R. Van Der Jagt, State University of Iowa. (Introduced by O. M. Helff.)
54. Integumentary pigmentation in the frog (R. pipiens) during metamorphosis, with especial reference to tail-skin histolysis. V. F. Lindeman, State University of Iowa. (Introduced by O. M. Helff.)
55. Tail atrophy of anuran larvae (R. pipiens) during metamorphosis, with respect to the growth of the urostyle. O. M. Helff, State University of Iowa.
56. Effect of prenatal and postnatal injections of the pituitary gland in the white rat. E. L. Corey, Yale University and Cold Spring Harbor Biological Laboratory. (Introduced by H. W. Stunkard.)
57. The effects of daily injections of bovine anterior-pituitary extract upon the developing albino rat. George E. Johnson and Everett D. Sayles, Kansas State Agricultural College.
58. The effect of mammalian follicular extracts on the oviducts of the frog (Rana pipiens Shreber). Opal M. Wolf, University of Wisconsin. (Introduced by F. L. Hisaw.)
59. Demonstration of the positive action of the placental hormone on the oviduct of fowls. Mary Juhn, University of Chicago, and R. G. Gustavson, University of Denver. (Introduced by F. R. Lillie.)
60. The lack of specific effects of endocrine substances upon Cladocera. L. A. Brown, State University of Iowa, and Arthur M. Banta, Carnegie Institution of Washington.
61. The effects of adrenalectomy in the lizard. A. Elizabeth Adams and Margaret Harland, Mount Holyoke College.
62. Studies on amphibian neoteny. I. Metamorphosis of the Colorado axolotl by the injection of inorganic iodine. W. R. Ingram, State University of Iowa. (Introduced by W. W. Swingle.)
63. The transplantation of ovary into capons. L. V. Domm, University of Chicago.
64. Transplantations of compensatory right gonad and normal rudimentary right gonad of the female brown Leghorn into cocks and capons. L. V. Domm, University of Chicago.
65. The effect of gamma radiation on the permeability of frog's skin to NaCl. Marvin M. D. Williams, University of Pennsylvania and Philadelphia General Hospital. (Introduced by J. H. Bodine.)
66. The effects of radium radiation on the oxygen consumption of grasshopper eggs. Reuben Blumenthal and Marvin M. D. Williams, University of Pennsylvania and Philadelphia General Hospital. (Introduced by J. H. Bodine.)
67. The specific conductivity of protozoan cultures. Elmer C. Herbert, Eleanor Slifer, Reuben Blumenthal, T. P. Sun, C. C. Wang, University of Pennsylvania. (Introduced by J. H. Bodine.)
68. Recovery of the heart beat of Fundulus embryos after stoppage by potassium chloride. Melvin W. Thorner, University of Pennsylvania. (Introduced by J. H. Bodine.)

69. The hydrogen-ion concentration of the contents of the alimentary tract of albino rats. Reuben Blumenthal, Elmer C. Herber, Eleanor Slifer, T. P. Sun, C. C. Wang, University of Pennsylvania. (Introduced by J. H. Bodine.)
70. Neutral red and salt antagonism in the Fundulus egg. J. H. Bodine, University of Pennsylvania.
71. The rate of killing of Daphnia magna with varying concentrations of mercuric chloride. John Breukelman and L. A. Brown, State University of Iowa. (Introduced by W. W. Swingle.)
72. Factors influencing water content and rate of metabolism in developing insect eggs (Orthoptera). J. H. Bodine, University of Pennsylvania.
73. Further study of the surface precipitation reaction. L. V. Heilbrunn.

### GENERAL EVOLUTION

*By demonstration*

74. The elimination of the male in the evolution of Cladocera. Arthur M. Banta, Carnegie Institution of Washington.
75. Polymorphism among the castes of termites. Alfred E. Emerson, University of Pittsburgh.
76. On the occurrence of a fresh-water medusa, Craspedacusta sp., in Stallworth Lake, Tuscaloosa, Alabama. William E. White, University of Alabama. (Introduced by John Y. Graham.)

*By title*

77. An hypothesis on the origin of graded series of local races in fishes. Carl L. Hubbs, University of Michigan.

### PROTOZOÖLOGY

*Read*

78. Heritability of the effects of chemically different media upon the fission rate within the clone of Paramecium caudatum. Austin R. Middleton, University of Louisville. (Lantern; 15 min.)
79. The biology of sewage disposal—a preliminary study. H. P. K. Agersborg, State of Illinois, Department of Conservation. (8 min.)
80. Relationships of the Opalininae. Charles A. Kofoid and Mary Louise Dodds, University of California.
81. The body size and the rate of fission in individuals of the protozoan Colpoda. Edward F. Adolph, University of Rochester. (Lantern; 12 min.)

*By demonstration*

82. Microkinematograph of life-history of Paramoecium. Charles S. Apgar, University of Pittsburgh. (Introduced by R. T. Hance.)

*By title*

83. Modifications of Chilodon uncinatus produced by ultraviolet rays. Mary Stuart MacDougall, Agnes Scott College.
84. Is fission the only method of reproduction in Amoeba proteus? P. L. Johnson, Johns Hopkins University. (Introduced by S. O. Mast.)
85. The pyrenoid of Chlamydomonas. J. McA. Kater, Utah Agricultural College.
86. On certain cytoplasmic inclusions in the flagellate, Peranema trichophorum. Richard P. Hall, University College, New York University.
87. A study of the metabolism of Euglena quartana. Woolford B. Baker, Emory University.
88. Relation of the volume of culture medium to growth rate of Euglena. T. L. Jahn, University College, New York University. (Introduced by R. P. Hall.)
89. The relation of density of population to rate of reproduction in Paramecium caudatum. Walburga A. Petersen, Memorial Hospital, New York. (Introduced by W. C. Allee).
90. The viability of Paramoecia and Euglenae in the digestive tract of cockroaches. Robert Hegner, Johns Hopkins University.

### COMPARATIVE ANATOMY

*Read*

91. Morphological studies of genetically different color patterns in Platypoecilus. Myron Gordon, Cornell University. (Introduced by the Secretary of Section F.)
92. Observations on the fat-bodies in Amblystoma maculatum. Gladys K. McCosh, Cornell University. (Introduced by the Secretary of Section F.)
93. Types of cysts, calcific concretions, and pigmentation on the full-term foetal placenta of the pig. Charles E. Abromavich, Jr., Johns Hopkins University. (Introduced by R. P. Cowles.) (Lantern; 7 min.)
94. Placentation of the Indian antelope (Antilope cervicapra). Charles E. Abromavich, Jr., Johns Hopkins University. (Introduced by R. P. Cowles.) (Lantern; 8 min.)

*By demonstration*

95. A new type of nephridia in nemerteans. W. R. Coe, Yale University.
96. Abnormal reproductive organs in Rana pipiens. Bernice L. Maclean, Mount Holyoke College. (Introduced by Ann H. Morgan.)
97. The leucocytopoietic organs of an Oligochaete, Pheretima indica (Horst). James E. Kindred, University of Virginia.
98. Ventral union in a bifid embryo of the earthworm, Sparganophilus eiseni. Florence Hague, Sweet Briar College.
99. Longitudinal sections of deer antlers, showing structure and growth during one season (Odocorleus virginianus). C. V. Noback, Heads and Horns Museum, Bronx Zoölogical Gardens. (Introduced by G. K. Noble.)

*By title*

100. The structure of the ductless glands in Alligator mississippiensis. Albert M. Reese, West Virginia University.
101. A new approach to the study of the ultimobranchial body. Amy Grace Mekeel, Cornell University. (Introduced by the Secretary of Section F.)
102. The pigment granules of human head hair: a comparative racial study. Leon Augustus Hausman, New Jersey College for Women (Rutgers University).
103. An investigation of the age correlations of two structural elements of human head hair. Elizabeth M. Wynkoop, New Jersey College for Women (Rutgers University). (Introduced by L. A. Hausman.)
104. The growth of the kidneys in the cat embryo. Homer B. Latimer, University of Kansas.
105. The development of the vertebral column of Anura and its bearing on the law of cephalocaudal differential growth. W. C. Senning, Cornell University. (Introduced by the Secretary of Section F.)
106. Sexual dimorphism of the symphysis pubis of the bat Myotis lucifugus (Miller). C. Hamre, R. K. Meyer, and S. J. Martin, University of Wisconsin. (Introduced by F. L. Hisaw.)
107. Studies in the anatomy and natural history of the devil-fish, Manta birostris. L. Hussakof, American Museum of Natural History.

## MISCELLANEOUS

*Read*

108. The indexed continuation card catalogue of the bibliography of fishes. E. W. Gudger, American Museum of Natural History. (10 min.)
109. The living organism and the new physics. Wm. G. H. Brehmer. (Introduced by the Secretary of Section F.)

*By demonstration*

110. A new staining dish for the handling of cover-slip preparations. Robert H. Bowen, Columbia University.
111. A paraffin oven for individual student use in courses involving histological technic. Robert H. Bowen, Columbia University.
112. Device for the control of temperature in localized areas of eggs and embryos. E. van Slyke, University of Pittsburgh. (Introduced by R. T. Hance.)
113. A method of obtaining blood directly from the branchial circulation of Necturus. Harry A. Charipper, New York University. (Introduced by A. B. Dawson.)

*By title*

114. Blue or black stains with haematoxylin. Elbert C. Cole, Williams College.

### CYTOLOGY

*Read*

115. Normal variations in red blood-cell counts and haemoglobin determinations. Christianna Smith, Mount Holyoke College. (Introduced by Ann H. Morgan.) (Lantern; 15 min.)
116. Connective-tissue structure of the ganglia of the earthworm. M. Thelma Holmes, Syracuse University. (Introduced by W. M. Smallwood.) (10 min.)
117. The membranous structure of striated muscle. H. N. Baker, H. C. McPhee, and Paul E. Howe, U. S. Department of Agriculture. (Introduced by the Secretary of Section F.) (Lantern.)
118. Further observations on the ovarian eggs of Fundulus. Mary J. Guthrie, University of Missouri. (12 min.)
119. Cytological studies on the spinning glands of Platyphylax designatus Walker (Trichoptera): respective rôles played by the nucleus and the Golgi apparatus during secretion. H. W. Beams and C. F. Wu, University of Wisconsin. (Introduced by George Wagner.) (Lantern; 7 min.)
120. Phenomena associated with the distribution of the plastidome in multinucleate cells of Ricinus. Robert H. Bowen, Columbia University. (Lantern; 10 min.)
121. Regeneration in nemerteans. W. R. Coe, Yale University. (Lantern; 12 min.)

*By demonstration*

122. The reaction of the 'segregation' apparatus of the leucocytes of the blood of Necturus to neutral red and to prolonged exposure to osmic acid. Alden B. Dawson, University College, New York University.
123. Hemocytopoiesis in the African lung-fish. H. E. Jordan and C. C. Speidel, University of Virginia Medical School.
124. A peculiar type of granulocyte in the spleen and thymus of the turtle. H. E. Jordan and C. C. Speidel, University of Virginia Medical School.
125. Connective-tissue structure of the ganglia of the earthworm. M. Thelma Holmes, Syracuse University. (Introduced by W. M. Smallwood.)
126. Hereditary rodless retina of the house mouse. Clyde E. Keeler, Bussey Institution.
127. The so-called secretory canaliculi of the human thyroid gland. Leslie B. Arey, Northwestern University Medical School.

*By title*

128. The chromosome cycle in the rotifer, Asplanchna amphora. David D. Whitney, University of Nebraska.
129. The rôle of the mitochondria in the formation of milk in the mammary glands of the albino rat. H. W. Beams, University of Wisconsin. (Introduced by George Wagner.)
130. The reaction of the 'segregation' apparatus of the leucocytes of the blood of Necturus to neutral red and to prolonged exposure to osmic acid. Alden B. Dawson, University College, New York University.

131. Yolk- and fat-formation in the egg of Patella vulgata, L. Hope Hibbard, Oberlin College.
132. The formation of 'pigeon milk.' H. W. Beams and R. K. Meyer, University of Wisconsin. (Introduced by F. L. Hisaw.)
133. Histological changes in the mucosa of the enteron of the snapping turtle, Chelydra serpentina, Linn., following prolonged inanition. Eileen M. Miller, New Jersey College for Women (Rutgers University). (Introduced by L. A. Hausman.)
134. Regeneration of the giant fibers in the earthworm. W. M. Smallwood, Syracuse University.

## EMBRYOLOGY

### *Read*

135. The cilia of the vertebrate oviduct in relation to the transpórt of spermatozoa. G. H. Parker, Harvard University. (10 min.)
136. A preliminary study of the function of the epididymis. William C. Young, University of Chicago and Brown University. (Introduced by Carl R. Moore.) (10 min.)
137. The origin of the first cleavage amphiaster in Echinarachnius eggs. Henry J. Fry, Washington Square College, New York University. (Lantern; 10 min.)
138. Early embryonic differentiation of the chick hypophysis in chorio-allantoic grafts. Kathryn Stein, University of Chicago. (Introduced by B. H. Willier.) (7 min.)
139. An experimental study of the origin of the adrenal gland in the chick embryo. B. H. Willier, University of Chicago. (Lantern; 8 min.)
140. Development of the thyreoid gland in Necturus maculosus. Waldo Shumway and James M. Sanders, University of Illinois. (10 min.)
141. The effect of the removal of median and lateral areas of the anterior end of the urodelan neural plate on the development of the eye. Howard B. Adelmann, Cornell University. (Lantern; 8 min.)
142. The differentiation of median and lateral areas of the anterior end of the urodelan neural plate when implanted into the belly wall. Howard B. Adelmann, Cornell University. (Lantern; 7 min.)
143. The growth of the entire body and of the limbs of Amblystoma larvae during heteroplastic parabiosis. Robert K. Burns, Jr., School of Medicine and Dentistry, University of Rochester. (Lantern; 15 min.)
144. Ultraviolet radiation as a means of twin production in Fundulus heteroclitus. Marie A. Hinrichs, University of Chicago. (Lantern; 10 min.)
145. Concerning the etiology of congenital polydactylism in the descendants of x-rayed mice. Halsey J. Bagg, Memorial Hospital and Cornell University Medical College. (Lantern; 10 min.)

### *By demonstration*

146. Insemination of starfish egg shown in motion pictures. Robert Chambers (assisted by C. G. Grand), Washington Square College, New York University.

147. Microdissection of starfish eggs shown in motion pictures. Robert Chambers (assisted by C. G. Grand), Washington Square College, New York University.
148. Human embryo, 36 mm. (hysterectomy case), vascular system injected with India ink within three hours after removal from uterus. Leon H. Strong, University of Michigan.
149. Variations in mouse embryos of eight days' gestation. Ezra Allen and E. C. MacDowell, Carnegie Institution of Washington.
150. Maturation in the parthenogenetic and sexual eggs of Moina macrocpa, a cladoceran. Ezra Allen, Carnegie Institution of Washington.
151. Parthenogenesis in lampreys; ova in male gonads of the brook and of the lake lamprey. Simon H. Gage, Cornell University.

*By title*

152. Germ-cell origin in the lake lamprey (Petromyzon marinus unicolor). E. O. Butcher, Cornell University. (Introduced by H. D. Reed.)
153. Origin of the germ cells in Amblystoma maculatum. Gladys K. McCosh, Cornell University. (Introduced by the Secretary of Section F.)
154. The development of fertilized fragments of the annelid egg. Edmund B. Wilson, Columbia University.
155. The effect of ultraviolet radiation on cleavage in the egg of Arbacia. R. A. Young, Howard University. (Introduced by E. E. Just.)
156. Development of the germ band and the embryonic envelopes of Diacrisia virginica Fabr. O. A. Johannsen, Cornell University.
157. Notes on a series of late embryos and newly hatched young of the chimeroid Callorhynchus. L. Hussakof, American Museum of Natural History.

### PARASITOLOGY

*Read*

158. A quantitative study of fowl coccidiosis. Benjamin P. Young, Cornell University. (Lantern; 15 min.)
159. A microscopical study of glochidial immunity. Leslie B. Arey, Northwestern University Medical School. (Lantern; 15 min.)

*By demonstration*

160. The life-history of Cryptocotyle lingua (Creplin). Horace W. Stunkard, New York University.

*By title*

161. The specificity of metazoan parasites for the host in its relation to the phenomena of age resistance and acquired immunity. J. H. Sandground, Harvard Medical School.
162. An acanthocephalid, Corynosoma strumosum (Rudolphi), in the California harbor seal. Gordon H. Ball, University of California at Los Angeles.

## ECOLOGY

### *Read*

163. The distribution of the Cyprinidae in the Great Lake Region. T. L. Hankinson, Michigan State Normal College. (Introduced by the Secretary of Section F.) (12 min.)
164. The distribution of varieties and species of Partula upon the islands of Tahiti and Raiatea. W. H. Longley, Goucher College. (Lantern; 12 min.)
165. The work of the University of Montana Biological Station at Flathead Lake. M. J. Elrod and R. T. Young, University of Montana. (Lantern; 15 min.)
166. A biological study of the Big Muddy River and its tributaries, polluted by domestic sewage and industrial wastes. H. P. K. Agersborg, State of Illinois, Department of Conservation. (8 min.)
167. Observations on certain littoral decapod crustaceans, with special reference to migrations from marine to terrestrial habitats. A. S. Pearse, Duke University. (Lantern; 10 min.)

### *By demonstration*

168. Factors determining the attachment of the cyprid larvae of the barnacle, Balanus galeatus. J. Paul Visscher, Western Reserve University.
169. On the formation of saliniclines in Barnegat Bay, N. J., with observations of the reactions of oyster larvae to salinity gradients. T. C. Nelson, Rutgers University.

### *By title*

170. The distribution and time of breeding of shrimps in Chesapeake Bay. R. P. Cowles, Johns Hopkins University.
171. Concerning the ecology of the muscid fly, Pyrellia serena Meigen, with special reference to hibernation. A. M. Holmquist, St. Olaf College. (Introduced by W. C. Allee.)
172. The effect of climatic and weather factors upon the number of birds on a depositing creek bank near Nashville, Tennessee. Jesse M. Shaver, George Peabody College for Teachers. (Introduced by W. C. Allee.)
173. Transformation sizes of the Ranidae of the United States and Canada. A. H. Wright, Cornell University.

# ABSTRACTS

## COMPARATIVE AND GENERAL PHYSIOLOGY

### *Read*

*1. Experimental study of spawning reactions of the female and male oysters.* Paul S. Galtsoff, U. S. Bureau of Fisheries. (Lantern; 10 min.)

The spawning reaction of the female oyster consists in rhythmical contractions of the adductor muscle and discharge of eggs. The reaction can be produced by the addition of a small amount of sperm to the surrounding water. The latent period of the reaction varies from a few minutes to half an hour. Neither the length of the latent period nor the intensity and duration of the reaction are dependent upon the amount of sperm added. After the reaction is over, the female for several days becomes immune to the sperm. The reaction takes place only at temperatures of 20°C. and above. Ultrafiltration experiments with sperm suspension show that the active principle does not pass through collodion membranes; it is very unstable and is destroyed by a temperature of 42° to 44°C.

A male oyster can be induced to spawn by the addition of eggs. The reaction consists in the discharge of sperm that flow with the stream of water produced by the gills. The latent period is very brief, the oyster responding to the stimulus almost immediately. The reaction can be repeated many times on the same oyster, which develops no immunity.

*2. Periodic reversal of heart beat in Lepidoptera.* John H. Gerould, Dartmouth College. (Lantern; 15 min.)

The direction of peristalsis of the dorsal vessel in the prepupa, chrysalis, and adult of Lepidoptera is not constantly forward, but phases either of backward peristalsis throughout the whole dorsal vessel or of diverging waves, originating in the third to fourth abdominal segments, proceeding backward and forward. *Silkworm.* Periodic reversal begins twenty-four hours after spinning, forty-eight before pupation. Pupation occurs during a vigorous backward phase, wholly backward, or in other individuals backward through the abdomen. Soon after pupation, backward phases consist of diverging waves. Later, and in the adult, complete reversal periodically occurs. Backward phases in the pupa are long (e.g., fifteen minutes), the average rate slow and variable, quickening at eight to ten days to that of the adult. Any long backward phase shows a gradual slowing down, except approaching pupation, when the pulse quickens to equal that of forward beating. The average rate of forward beating is constant at constant temperature, but the absolute rate in a long phase gradually slackens. Pauses (sometimes one and one-half hours long in older pupae) often follow forward phases. Alternating phases in the adult, compared with the pupa, are short, usually less than one hundred beats. In old moths the phases become still shorter, often reduced to single beats. Then, and at other reversals, opposing waves occasionally conflict. Periodic reversal occurs for hours after the dorsal wall with pericardium and heart has been excised. As in ascidians, back pressure cannot explain reversal.

3. *X-ray effects in relation to physiological condition in Paramoecium.* Robert T. Hance, University of Pittsburgh. (Lantern; 5 min.)

X-radiation affects the permeability of paramoecium under certain conditions. Variable results were obtained when the x-rayed specimens had been taken from mass cultures. When, however, animals of known ages were used, consistent increase or decrease of the permeability followed exposure to x-ray, depending on the age of the cell at the time of radiation.

4. *The ionization and voltage of x-rays in relation to biological reactions.* Robert T. Hance, University of Pittsburgh. (Lantern; 5 min.)

Experimental studies on mice and paramoecium have demonstrated the similarity of their reactions to hard and soft x-rays when the exposures are of such duration as to produce an equivalent ionization in each case. This is in agreement with physical theory, although contrary to some clinical experience.

5. *Behavior of amebas in a magnetic field.* A. A. Schaeffer, University of Kansas. (Lantern; 15 min.)

The character of movement of several species of amebas was observed while they were moving in glass tubes in a strong electromagnetic field. The tubes containing the amebas were immersed in water in a narrow glass trough which also held the bulb of a small thermometer. The temperature was maintained at a very nearly constant level by cooling off the large pole pieces with pans of cold water. In most of the experiments magnetic and open fields were alternated many times in each experiment.

The general effect of the conditions of the magnetic field is: 1) an increased rate of movement, which is much more pronounced in amebas that have become sluggish after long confinement in the tubes; 2) a markedly decreased amount of fluctuation in the rate of movement in successive spiral turns during the existence of the field and also afterward.

6. *The persistence of an instinct in Microstomum caudatum.* Wm. A. Kepner and J. W. Nuttycombe, University of Virginia. (Lantern; 10 min.)

The senior author has reported that Microstomum appropriates, for use, the nematocysts of hydra, upon which it will feed when the rhabdocoele lacks a full complement of nematocysts. If Microstomum has a full complement of nematocysts at its surface, it will eat hydra only after long inanition. In this case the nematocysts of hydra will be rejected.

In this is seen a complex instinct of Microstomum. We have undertaken to determine whether the instinct may be carried through frequent generations that have had no experience with hydra.

We are able to report that we have carried Microstomum through twenty-two generations, none of which have had any experience with hydra. After feeding the successive generations, as they appeared through fission, upon oligochaetes and tadpole brain for twenty-two successive generations, it was found that an individual of the twenty-third generation, when presented with a hydra, accepted it and appropriated the nematocysts. Thus in Microstomum we have a complex instinct persisting through twenty-two generations, in none of which the instinct has been operative. The middle third of an individual—sixteen generations removed from experience with hydra—when it had regenerated anterior and posterior regions, accepted hydra and appropriated its nematocysts.

7. *The effect of light on the eyes, pigmentation, and behavior of the cave salamander, Typhlotriton.* G. K. NOBLE AND SARAH H. POPE, American Museum of Natural History. (Lantern; 15 min.)

Larvae of Typhlotriton spelaeus have normal eyes; adults have degenerate retinas and eyelids fused, except near the center where they usually overlap. Rearing of thyroid-treated larvae under various conditions shows that eye degeneration is due to absence of light. Fifty-eight young, exposed to diffuse light at 19°C. average temperature from 35 to 345 days after skin shedding, have open lids. Twenty-five controls in darkness show all stages in the fusion of lids. Four young, retained in diffuse light at 11.5°C. from 52 to 143 days after shedding, have open lids; twenty-seven others, in darkness at the same temperature from 61 to 338 days after shedding, show all degrees of lid fusion. In experimentals with open lids, retinas are distinctly degenerate. Recently metamorphosed young from caves have open lids, and one large, sexually mature male was found in nature with open lids and complete retina.

In direct light the melanophores of adults expand, but not those of larvae. Adults exposed to light for 215 days show no increase of pigmentation nor opening of the lids, while the pigmentation is distinctly increased in young exposed for 182 days after shedding.

Larvae are negatively heliotropic, but are found chiefly near the mouths of caves. Their distribution seems limited by available cold water, for their tolerance is lower than that of the wide-ranging Eurycea multiplicata.

8. *Further notes on color changes induced in fishes by light rays of varying wave length.* ELLINORE H. BEHRE, Louisiana State University. (10 min.)

It has been suggested that the color changes in fishes under various light filters, reported before this group last year, might have been due to differences in total radiant energy transmission rather than to differences in the wave length of the transmitted light rays. To test this, a new series of checks was made with the same light filters previously used, restandardized, and calibrated for total energy transmission.

The results were of the same order as the earlier experiments, but less marked. Blue was still the strongest fading factor, minus blue the strongest darkening factor; but the difference between the effect of any two colors was reduced, indicating that a part, though only a part, of the effect produced was due to total radiant energy transmission.

*9. The rate of dark adaptation in the eye of Eristalis tenax—a preliminary report.* William L. Dolley, Jr., University of Buffalo. (Lantern; 15 min.)

The rate of dark adaptation in the eye of Eristalis tenax was measured in the following manner. One eye of a given specimen was dark-adapted for various lengths of time. During the same periods the other eye was exposed to light of known luminous intensity. The insect was then placed at the intersection of two horizontal beams of light of equal luminous intensity which crossed at right angles. Under these conditions, the degree of deflection of the path of the organism from the diagonal bisecting the angle formed by the beams depends upon the degree of dark adaptation in the one eye. Taking advantage of this fact, it was found that, upon exposure to darkness, sensitivity to light in the eye of Eristalis tenax increases to a maximum of about twenty-one times its initial sensitivity in about sixty minutes, remains at about the same level for the following two hours, but in the next hour decreases rapidly.

*10. Chroma-vision in the honeybee.* L. M. Bertholf, Western Maryland College. (Introduced by S. O. Mast.) (Lantern; 15 min.)

Chroma-vision is defined as the ability to differentiate diverse chromas made equal in brilliance to the animal in question. Hence, the problem is, first, to ascertain the relative brilliance to honeybees of a series of chromas, then ascertain by tests whether or not the bees can differentiate these when set at equal brilliance. Five color filters were chosen, four 'Corning' glasses and one cell containing potassium-dichromate solution, each used in connection with a cell containing copper-sulphate solution. The relative brilliance of each was ascertained by equating the stimulating effect on bees of a beam of light passed through each filter to that of a variable white beam. Then training tests were made, using two chromas at a time, as follows: Bees entered a dark room and walked into the intersection of two differently chromed beams of light crossing at right angles; food could be obtained only by walking down one beam, not the other; most of the bees, after a day or two, came to orient correctly; then the position of the two beams was suddenly exchanged, upon which a significant percentage of the bees exchanged their route correspondingly. In twenty-one experiments, using six different combinations of chromas, it was established not only that bees possess chroma-vision, but that they are able to differentiate violet, blue, green, and red. The addition to this list of blue-green and ultraviolet, as found by Kühn, gives six chromas claimed at present to be differentiable by bees.

*11. The effect of dislocation of the eye and of unilateral blinding on the orientation and equilibrium in fish.* Theodore Koppányi, Syracuse University. (15 min.)

Several species of fish were used in these experiments. Unilateral blinding, if performed with suitable technique and under ether anesthesia, did not produce circus movements or pronounced tilting toward the uninjured side (contrary to Lyon, '09, and Main, '28). Even normal and totally blind fish, especially goldfish, very often tip the anteroposterior axis (about 5° to 25°).

The effects of unilateral blinding can be in no way compared with those of dislocation of one eye and removal of the other. Dislocation of the eye from its natural orbit into an artificial one on the top of the head produces a constant and very marked tilting of about 45° to the vertical.

12. *The olfactory organs in the meal-worm beetle, Tenebrio molitor.* J. M. Valentine, Western Reserve University. (Introduced by J. Paul Visscher.) (10 min.)

T. molitor was found to be exceptionally well fitted for olfactory experimentation: it long survived mutilation of the antennae; the sexual reactions of the male were energetic and highly characteristic. Olfaction was discovered to be the sole means of communication between the isolated male and female. Antennal mutilation showed that the olfactory organs operative in sexual reactions were located only on the antennae, and particularly on their four distal segments. This was subsequently confirmed through a microscopic examination of the antenna which brought to light large aggregates of peg organs on these segments. Micro-injection of the antenna gave evidence of a permeable peg-organ cuticle. Experiments such as have been performed by recent workers, who have used essential oils and who have discovered organs scattered over the body sensitive to these reagents, were repeated with T. molitor. The results were incompatible with those obtained with the extracted odor of the female. It was concluded that the sex stimulus was a true odor, and that strong essential oils acted in a manner suggesting an irritant. A difference in the reactions to the two classes of stimuli was demonstrated by beetles deprived of one antenna.

13. *The effect of the injection of thyroid extract on the polynuclear count in a perennibranchiate amphibian (Necturus maculosus).* Harry A. Charipper, New York University. (Introduced by Alden B. Dawson.) (Lantern; 10 min.)

Intraperitoneal injection of thyroid extract affects the blood tissue of Necturus maculosus. The normal polynuclear count for this amphibian is shown to be similar to that found in man and rabbit. Intraperitoneal injection of a filtered saline extract of thyroid substance (Armour's) affects this count. The percentage of single-lobed, class-I, polymorphonuclear neutrophilic leucocytes steadily increases and reaches a maximum within twelve hours after injection. There is a proportional decrease in the percentage of the other classes of neutrophilic cells. After three days, the number of bilobed, class-II, polymorphonuclear neutrophiles shows an increase, while the class-I cells tend to approach the normal. After nine days, the polynuclear count is approximately normal. This left deflection of the polynuclear count is found to be directly proportional to the dosage (milligrams of thyroid substance per gram body weight). Attention is directed to the fact that injection of thyroid produces similar effects in the mammal and the amphibian, irrespective of the location of the granulocytopoietic centers. Further, it is of interest to note that the reaction of the blood tissue of Necturus, as shown by the present work, is the only reaction to thyroid thus far reported for any tissue of this amphibian. The method for obtaining blood for this study, directly from the branchial circulation, will be presented as a demonstration.

14. *The effect of thyroxin injection on the hair color, hair growth, and thyroid-gland structure in Peromyscus.* Ralph R. Huestis and Harry B. Yocum, University of Oregon. (Lantern; 10 min.)

In an attempt to discover whether or not differences in color of two subspecies of Peromyscus, one living along the Oregon coast, the other in the high arid region, might be due to differences in the thyroids, as we reported a year ago, a series of inland mice were injected with thyroxin. Each mouse received 0.05 mg. thyroxin in physiological salt solution every five days. A similar group was selected as controls. The hair from the right side of each mouse was removed by means of depilatory paste. The animals were killed when the regenerated hair had reached the length of the hair on the opposite side. Samples of hair from similar regions on both sides were taken and percentages of black and yellow hairs obtained. Sections were made of the thyroids. The following conclusions have been reached: 1) Hair is regrown more rapidly on injected mice, as is evidenced by the fact that, of the first fourteen mice killed when their hair had regenerated, ten were from the injected group. 2) On the depilated side in both groups the regenerated hair was more sparse, with a higher percentage of black hairs. There was thus no evidence that thyroxin had increased pigmentation. 3) Comparisons of the thyroids indicated that thyroxin had affected those of the injected group, while those of the controls were unchanged. Thyroid follicles of the former group were larger and more numerous, thus resembling thyroids of coast mice, while thyroids of the controls were typical for mice from eastern Oregon.

### *15. Comparative metamorphogenic properties of three iodized isomeres of tyrosin (di-iodo-paratyrosin, di-iodo-metatyrosin, and di-iodo-orthotyrosin) on R. temporaria larvae.* **ALEXANDER B. GUTMAN**, Cornell University and University of Halle. (Introduced by H. D. Reed.) (12 min.)

Paratyrosin was obtained by hydrolysis of silk waste and iodized. Meta- and orthotyrosin were synthesized according to the method of Blum and iodized for the first time. Determinations by the macrocarius method proved all three compounds to have the same iodine content; differing only in the relationship of the alanin radical of the OH group. Then 0.5 cc. of a 1 per cent solution of each isomere was administered to eight groups of twenty specimens each and renewed every second day, the specimens used being Rana temporaria tadpoles, averaging 1.6 cm. in total length. Those treated with the ortho form were all dead in ten days with extreme thyrotoxis symptoms; those treated with the para form evinced definite metamorphic changes in two weeks and were dying rapidly in four weeks with toxic symptoms; the meta-treated specimens could not be distinguished from the controls after five weeks. A second series, averaging 2.6 cm., treated with half the dosages gave similar results, the meta-treated specimens remaining at early metamorphic stages. The experiments indicate that iodine content is not an index to the metamorphogenic properties of a substance and give an insight into the iodine metabolism of the metamorphosing amphibian.

### *16. The biological significance of amphibian parathyroids.* **ROY A. WAGGENER**, Cornell University. (Introduced by the Secretary of Section F.) (12 min.)

The functional significance and the vital importance of the parathyroid glands have been quite satisfactorily demonstrated in the case of the higher vertebrates, particularly the mammals, where parathyroidectomy was followed by a series of profound effects upon the organism. These little bodies are believed to be absent in the fishes. They put in their appearance at some stage in the life-cycle of most amphibians and have been found in most, if not all, higher vertebrates which have been investigated up to the present time. It was thought desirable to test the importance and physiological effects which these small clumps of cells exhibit in this conservative group of amphibians. The experimental method was employed and some of the results of parathyroidectomy, blood-calcium determinations, Para-thor-mone injections, and bleeding, on Rana catesbeiana (Shaw) are submitted in this paper. An attempt is also made to point out the bearing of these studies upon two of the major aspects of the whole parathyroid problem, namely, the biological significance of the parathyroids and their physiological importance in general.

### 17. *Hypophysectomy in the brown Leghorn fowl.* J. B. MITCHELL, JR., University of Georgia. (Introduced by F. R. Lillie.) (Lantern; 10 min.)

This study concerns the effects produced in the brown Leghorn fowl by the operative removal of the anterior lobe of the pituitary gland. Young birds, the majority from three to seven weeks old, were operated by the oral route, under ether anaesthesia, the gland removed by negative pressure, and in some cases the site cauterized (electric cautery). Controls of the same age were, 1) normal unoperated birds and, 2) birds having the same operation as for removal, but with the gland left intact, or disturbed but not removed. Cautery was sometimes omitted as a check on its effect. Birds were carefully watched, periodically inspected and weighed. At autopsies, thyroids, parathyroids, adrenals, and gonads were fixed for microscopic examination. The hypophyseal site was removed in toto, fixed, decalcified, and serially sectioned to determine the extent of glandular destruction and brain injury.

Complete removal of anterior lobe was found to be fatal within about ten days, despite apparent recovery from operation. Partial removal was not fatal, despite frequently greater traumatization and hemorrhage. If the remaining fragment was very small, the birds were distinctly retarded in growth. There was no specific inhibition of the genital system, though sex maturity and adult plumage were delayed in proportion to general retardation. The thyroids of these birds were reduced in size and filled with colloid to a marked degree above normal. Parathyroids and adrenals showed no special changes. One hundred and ninety-two birds were used, one hundred and sixty-one hypophysectomized and thirty-one controls.

### 18. *Effect of anterior-lobe substance on basal metabolism in salamander larvae.* SAUL SCHWARTZBACH AND EDUARD UHLENHUTH, University of Maryland Medical School.

In previous work we found that intraperitoneal injection of anterior-lobe substance (Armour's commercial anterior-lobe powder as well as fresh gland collected and desiccated by ourselves) induces precocious metamorphosis of salamander larvae and forces the thyroid gland into functional activity. In thyroidectomized larvae anterior-lobe substance is completely ineffective. In accordance with its activating effect upon the thyroid is the power of anterior lobe to raise the basal metabolism.

The basal metabolism of normal and of injected larvae (eastern as well as western race of Amblystoma tigrinum) was tested by measuring the oxygen consumption.

The experimental larvae consumed about 40 per cent more oxygen than the control larvae. Four injections sufficed to raise the oxygen consumption; but increasingly more oxygen was consumed with increasing number of injections. The maximum was reached at the time at which the display of the functional phase of the thyroid gland (collapse of follicles, secretion vacuoles in the cells) attained its maximum.

A rise in basal metabolism has been reported by Cushing and his coworkers in human acromegalics and by P. E. Smith and his coworkers in mice after the injection of anterior-lobe substance.

We conclude from our experiments that anterior-lobe substance raises basal metabolism indirectly by forcing the thyroid gland into excessive activity.

*19. The effects of subcutaneous injections of extracts of wolffian body on the growth and sexual development in the albino rat.* C. D. DAY, Kansas University. (Introduced by H. H. Lane.) (15 min.)

Certain conditions suggest an endocrine, rather than a renal, function for the mesonephroi of the Eutheria. With this idea in mind, rats were given subcutaneous injections of extracts made from the wolffian bodies of pig fetus. Directions are given for securing wolffian bodies and the preparation and administration of the extracts, of which there were two kinds, unheated and heated. Summaries of very detailed observations on growth, development of the scrota and penes, relationship to growth and testicular position, fur, and behavior are given. Graphs representing individual growth and group growth above original weight are given. Graphs showing changes in testicular position from week to week are also given. The results of the experimentation would seem to warrant the following conclusions: 1) The wolffian body contains two principles, one of which accelerates growth, the other sexual development. 2) The principle having to do with body growth is partially removed or destroyed by heating at 90°C. for three minutes. 3) The principle having to do with sexual development is entirely removed or destroyed by heating at 90°C. for three minutes. 4) The general well-being of animals receiving subcutaneous injections of the unheated extract is slightly augmented; similar injections of the heated extract exert no influence in this respect. 5) Neither the unheated nor the heated extracts when injected subcutaneously have any effect on the fur of the rat.

*20. The production of hypertrophy of the müllerian ducts in castrated males of Triturus viridescens by ovarian grafts.* A. ELIZABETH ADAMS, Mount Holyoke College. (Lantern; 15 min.)

Fourteen adult male Triturus viridescens were castrated in the fall (October 18 to November 11, 1927) and ovaries (in some cases with fat-bodies) of adult females were placed in the body cavity. Three of the animals died within one or two days after the operation, but eleven lived from 200 to 292 days, when they were killed. In each of these was found ovarian tissue varying in amount, degree of integrity, and vascularization. The vascular connections had usually been established with liver, body wall, or mesentery, occasionally with other structures. In the normal male the müllerian duct is a very rudimentary structure lying at the side of the wolffian duct and often visible only in sections.

In all of the experimental animals the müllerian ducts had hypertrophied (in eight markedly so) and could be seen parallel to the wolffian ducts and anterior to the kidneys. Gross observation and study of sections of the ducts indicated secretory activity of the cells lining the ducts. The degree of hypertrophy appeared to be dependent upon the amount of ovarian tissue present, and it seems clear that a hormone of the transplanted ovary is responsible for the enlargement of the rudimentary müllerian duct of the male. The wolffian ducts in the animals with ovarian grafts had atrophied as usual after castration, but they were noticeably dilated with a yellowish fluid, although their cells resembled those of a typical castrate.

*21. The effects of early ovariotomy in the brown Leghorn fowl.* L. V. Domm, University of Chicago. (Lantern; 15 min.)

The work of Brode ('28) has made it highly probable that no primordial germ cells were present in the right rudimentary gonad at the time of ovariotomy in our earlier series (Domm, '24, '27). Hence it was considered advisable to supplement this otherwise extensive series by additional cases of early ovariotomy.

Many females were ovariotomized, ranging from one to fifty days in age. Following complete ovariotomy, the right gonad forms an organ of testis-like form and structure. In spite of the early age at operation there has been no indication of the occurrence of spermatogenesis in the fifty-six cases so far studied. Observations indicate that the rate of growth of the right rudimentary gonad is slow, so that in a bird ovariotomized during the first week it may not attain an appreciable size until the bird is approximately two to three months old. On the contrary, birds ovariotomized any time after three to four months may show a very rapid development of this gonad. The juvenile plumage of the male develops after ovariotomy, corresponding approximately in time of appearance with that of the male of the same age. Many later developed adult male plumage, but ultimately reverted to female plumage. Others never develop adult male plumage, but adult female follows directly on juvenile male. These variations in plumage are correlated with the rate and extent of growth of the right compensatory gonads. In other respects, relating principally to the assumption of male secondary sex characters, these cases are similar to those described in our former series.

*22. Antibody formation against an animal's own tissue.* Paul S. Henshaw, University of Wisconsin. (Introduced by L. E. Noland.) (10 min.)

It has been possible to cause an animal to form antibodies against its own skin-tissue proteins as well as against other foreign-tissue proteins. As recognized by the precipitine test, skin may be destroyed on a rabbit's ear with ultraviolet light sufficient to send an active antigen into the blood stream, and thus have an antibody formed against skin tissue of the animal's own body. In like manner, guinea-pigs may be sensitized by severely burning the skin on the back and sides with ultraviolet light, so that when injected with skin solution mild anaphylaxis results.

Similar actions caused by trauma, sunlight, or other environmental influences may account for hypersensitivity in anaphylaxis and skin hypersensitiveness, as described many times. As yet, no attempts have been made to determine the specificity of skin-protein solution or to increase the intensity of this reaction.

*23. Some effects of crowding, pollution, food, and other factors on growth of the pond snail, Lymnaea stagnalis appressa.* EDWARD D. CRABB, University of Pennsylvania. (Lantern; 10 min.)

Two methods of rearing snails were employed in this work: *a*) mass cultures in which the number of snails and quantity of water were varied, and, *b*) isolation cultures in which individuals were reared in a finger-bowl containing 150 to 175 ml. of medium. The continued presence of excrement or decaying desiccated gland tissues in the media, marked crowding, insufficient food, or calcium-free media markedly retard growth. If individuals thus stunted are isolated in finger-bowls of tap-water before reaching an age exceeding 125 to 150 days, fed leaf lettuce, filter-paper, and boiled wheat grains, and kept in a well-lighted room at about 20°C., the excrement siphoned off, and fresh water added every two or three days, they may be expected to grow exceedingly rapidly and ultimately attain the size of individuals reared under such conditions since birth. In a small percentage of these isolated controls growth is unaccountably retarded, but when about two months old these begin to grow very rapidly, as do those which have been artificially stunted.

*24. The nutritional requirements of brook trout and their rate of growth.* C. M. MCCAY, W. E. DILLEY, AND M. F. CROWELL, Cornell University and Connecticut State Hatchery. (Introduced by the Secretary of Section F.) (Lantern.)

Experiments to define the fundamental nutritional requirements of brook trout have been extended. Experimental groups have been stunted for periods of twenty-two weeks upon low-protein diets without losing their ability to grow normally. When trout are allowed to grow for periods of one month with alternate periods of stunting for the same length of time, they attain an accelerated growth rate that tends to compensate for the periods of stunting. Trout allowed to grow upon purified rations with an adequate content of protein live only half as long as those stunted upon the same rations with a low protein level.

Trout grow quite normally for a period of several months upon a ration of dry skim milk, but ultimately die. Skim milk supplemented with raw liver permits brook trout to attain a maximum growth rate which is quite superior to that obtained upon liver alone. The growth rates of trout over a period of one year have been determined. Measurements of the food requirements in calories to produce a given increase in body weight show that the trout is as efficient a converter of energy as the best of the higher vertebrates. Trout are able to employ the cereal grains for both growth and maintenance. The unknown constituent of raw meat which permits trout to grow differs from any of the known vitamins. It is not extracted by ether or alcohol. It is not the same constituent which is responsible for blood regeneration in higher mammals.

*25. The effect of lethal concentrations of salts on fish.* R. T. YOUNG, University of Montana. (Lantern; 15 min.)

Several species of fresh-water fish (Perca flavescens, Ameiurus nebulosus, Pimephales promelas, and Carassius auratus) subjected to lethal concentrations of various salts ($NaCl$, $MgSO_4$, $CaCl_2$, and $KCl$) in some cases show no evident pathological effects of the treatment. In other cases there is distinct congestion with epithelial necrosis. These changes are most evident in the gills, but are also present to some extent in liver, kidney, and intestinal tract. Counts of the red corpuscles give no constant results. In some cases their number is diminished; in others, very greatly increased. It is probable that the pathological changes are the result or accompaniment of the lethal factor or factors, rather than the cause of death in themselves.

*26. The effects of divalent and monovalent cations on locomotion in Amoeba proteus.* DWIGHT L. HOPKINS, Duke University. (Lantern; 15 min.)

The effects of divalent cations were studied by adding them in the form of their chlorides in increasing concentrations to potassium phosphate buffer pH 6.6 having a potassium concentration of 0.00678 N., and obtaining the rate of locomotion of amoebae in each of the solutions of the series. The effects of monovalent cations were studied in the same way, except that they were added to calcium phosphate buffer pH 6.6 having a calcium concentration of 0.00678 N.

It was found that amoebae would not locomote in either of the buffers alone, but that the addition of small amounts of monovalent cations to the buffer containing monovalent cations allowed for locomotion. Li, Na, K, and Rb antagonize calcium. Ca and Sr antagonize potassium. Mg and Ba will not antagonize potassium. Potassium is more effective in antagonizing calcium when it is in combination with chlorine than when it is in combination with phosphate. The same statement holds for calcium antagonizing potassium. The efficiency of the different monovalent cations in antagonizing calcium is in the order: $Na>K>Li>Rb$. The efficiency of the different divalent cations in antagonizing potassium is in the order: $Ca>Sr>Mg=Ba$.

*27. Fertilization of marine ova in dilute sea-water and its significance for some problems in cell physiology.* E. E. JUST, Howard University. (Lantern; 15 min.)

Ova of various marine animals are capable of fertilization in dilutions of sea-water (as the tables show) with a high degree of normal development. On return from the dilutions to normal sea-water, the ova form temporary vacuoles. This finding is considered important for three reasons: 1) It gives some hint concerning the distribution of water in protoplasm and therefore concerning protoplasmic structure. 2) It assigns a rôle to definite cellular structures in the hydration of protoplasm. 3) It is of value for the study of cell permeability.

*28. Anaesthesia and cell division in sea-urchin eggs.* REUBEN BLUMENTHAL, Marine Biological Laboratory and University of Pennsylvania. (Introduced by J. H. Bodine.) (Lantern; 5 min.)

That there is a rhythm of susceptibility during the cleavage cycle of sea-urchin eggs has been satisfactorily demonstrated. The question as to whether the greatest susceptibility occurs immediately before or immediately after cleavage, is not, however, so definitely settled. It is well known that anaesthetics will prevent cell division. In the present series of experiments, however, it was found that certain anaesthetics, even in very high concentrations, will not prevent cell division of Arbacia eggs if the eggs are exposed to the anaesthetics within ten minutes of the time when cleavage in normal eggs is due. This highly resistant period occurs before every cleavage, but the length of the period decreases with every cell division. Eggs which have cleaved once in the anaesthetic will not cleave again if permitted to remain in the anaesthetic solution. Cleavage which takes place in the anaesthetic occurs in exactly the same time as cleavage in the normal eggs. The anaesthetics used were: iso-amyl, capryl, iso-propyl, n-propyl and methyl alcohols, ethyl nitrate, ethyl acetate, and potassium cyanide. All these anaesthetics were used in concentrations which normally inhibit cell division. In only one solution was cell division prevented if eggs were placed in it within ten minutes of their cleavage time. This occurred in the case of methyl cyanide.

29. *Specific effects of low temperature on marine egg cells.* NELLIE M. PAYNE, University of Pennsylvania. (Introduced by J. H. Bodine.) (Lantern; 5 min.)

In eggs of Arbacia punctulata temperatures from 0°C. to 5°C. increase protoplasmic viscosity, decrease elasticity, and lower the iodine number of the fat. In eggs of Cumingia tellinoides, elasticity of protoplasm is decreased. Cleavage of egg cells takes place, but individual micromeres tend to fall apart. Micromeres can continue to divide after separation from the cleavage mass. Addition of $CaCl_2$ in amounts sufficient to just double the Ca of sea-water prevents micromeres from falling apart.

30. *The effect of acids and alkalies on the viscosity of Arbacia-egg protoplasm.* L. G. BARTH, University of Chicago. (Introduced by L. V. Heilbrunn.) (Lantern; 15 min.)

The effect of various acids and alkalies at varying pH on the viscosity of Arbacia eggs was determined by using the centrifuge method. The relative value of the viscosity at various H-ion concentrations was found for a number of different acids. A comparison was made of the action of acids and alkalies in NaCl and in sea-water. Under the conditions of permeability in sea-water, the acids have markedly different effects, the weakly dissociated acids in general producing gelation at a higher pH than the strongly dissociated ones. When acids were dissolved in NaCl, no differences in their action on viscosity could be detected. When alkalies are dissolved in sea-water, a weakly dissociated base ($NH_4OH$) produces a decrease in viscosity which reaches a maximum at 9.6, while a strongly dissociated base (NaOH) produces no effect. At pH 10, $NH_4OH$ produces a coagulation of the protoplasm. If NaOH be dissolved in NaCl, however, it produces a viscosity decrease similar to that caused by $NH_4OH$. The most probable explanation of the effect of NaCl is that it increases permeability, allowing strongly dissociated substances to penetrate the cell membrane. At a pH of 5, any acid will produce a gelation of the protoplasm if it is able to penetrate. The gelation is due to an increase in the viscosity of the ground-substance or the material of the hyaline zone which is produced by centrifuging. The decrease in viscosity is likewise due to a decrease in the viscosity of the ground-substance.

*31. The rate of swelling of Arbacia eggs in solutions of ammonium salts.* DOROTHY R. STEWART, University of Pennsylvania Medical School. (Introduced by M. H. Jacobs.) (7 min.)

To throw light on the mechanism of the penetration of cells by certain hydrolyzed salts, the rate of swelling of Arbacia eggs has been studied in solutions of the ammonium salts of five of the lower fatty acids. In pure solutions of the salts isosmotic with sea-water the rate of swelling is in the order: valerianate>butyrate>propionate>acetate>formate. In solutions of the salts made up in bicarbonate-free sea-water or in M/2 KCl the same order is found for the recovery of the original volume after preliminary shrinkage. The increase in volume is more rapid at pH 6.5 than at pH 7.0 and at pH 7.0 than at pH 7.8. No significant change in volume occurs in solutions of the ammonium salts of strong acids. However, cells exposed to such solutions for an hour and a half are still capable of swelling in solutions of ammonium acetate. There is some evidence that fertilized eggs swell more rapidly than unfertilized.

*32. Potential differences across the chorion of the Fundulus egg.* MARGARET SUMWALT, University of Pennsylvania Medical School. (Introduced by M. H. Jacobs.) (8 min.)

Difference of potential between the inside and the outside of the chorion of single fertilized eggs of Fundulus heteroclitus was measured. Its magnitude and sign varied with the concentration and composition of the solution in which the egg was immersed. These variations were consistent qualitatively, in neutral solutions, with those described by Michaelis for dried collodion membrane models; and, interpreted analogously, furnished evidence that the chorion exhibits a differential permeability to cations and anions. In neutral solutions it is more permeable to cations than to anions, and the penetrating speeds of different cations are in the same order as their mobilities in free diffusion. As the H-ion concentration of solutions is increased, the ratio of anion to cation permeability is increased and finally inverted. With KCl in the concentrations used, the pH at which it reaches a value of 1 is about 3.7.

*33. The influence of oxygen tension on the oxygen consumption of marine fishes.* F. G. HALL, Duke University. (Lantern; 12 min.)

Three types of marine fishes were studied: the scup, which is very active; the puffer, which is less active; and the sluggish toadfish. There appears to be a correlation between the activity of fishes and the influence of oxygen tension on their oxygen consumption. There is apparently a direct relation between hemoglobin concentration and activity, the more active fishes having the higher concentrations.

*34. The effect of temperature on the rates of oxygen consumption in insects.* EDITH ROGERS, University of Pennsylvania. (Introduced by J. H. Bodine.) (Lantern; 5 min.)

One hundred and eighteen grasshopper nymphs between the second and fourth instar were used separately and the effect of temperature on their oxygen consumption noted. The measuring instruments were modified Krogh manometers. Temperatures ranged from 10°C. to 52°C.

Increase in temperature caused an increase in the rate of oxygen consumption. Nymphs exposed to single temperatures gave consistently higher rates of oxygen consumption than nymphs exposed to a series of temperatures. The values for the temperature coefficient, $Q_{10}$, decreased with increase in temperature. The data do not agree with Krogh's theory for the effect of temperature on physiological reactions. Critical thermal increments have been calculated and found to be of two types: $\mu = 11{,}300$ and 16,840; above 30°, the first value is obtained, the second below this temperature.

### 35. *Effect of acidosis in strychnine poisoning.* W. F. Wenner and E. W. Blanchard, State University of Iowa. (Introduced by W. W. Swingle.) (5 min.)

It has been demonstrated that changes in the reaction of the blood toward the acid side prevent the spasticity of the skeletal muscles in parathyroid tetany. Recent experiments show that a decrease in the pH of the blood produced prior to the injection of lethal doses of strychnine prevents violent tetanus, convulsions, and death.

Acidosis was produced in one series of dogs by administering $CO_2$ and $NH_4Cl$ and in another series by subcutaneous injections of uranium nitrate and sodium tartrate. When the pH of the blood dropped to about 7.2 and the $CO_2$ capacity to about 22 volumes per cent, the dogs received subcutaneously $\frac{1}{160}$ gram per kilo of strychnine sulphate. Ten minutes later, slight muscle tremors and spasms developed. In all cases recovery occurred within forty-five minutes. One dog that had received an unusually large dose of uranium nitrate several days previous to the injection of strychnine died one hour later. Death in this case was apparently due to uranium-nitrate poisoning. Control dogs receiving the same lethal dose of strychnine died in violent convulsions within one hour.

From our results it is evident that it is the initial drop in pH occurring before injection of strychnine, and not the fall produced by convulsions and the resulting anoxemia, that is effective in preventing convulsions and death from strychnine poisoning.

### 36. *Effect of intravenous injections of alkali on the physiological action of curare.* W. F. Wenner and E. W. Blanchard, State University of Iowa. (Introduced by W. W. Swingle.) (5 min.)

In another series of experiments we have shown that a decrease in the pH of the blood produced prior to the administration of lethal doses of strychnine prevented violent tetanus and death. It was considered worth while to test the reciprocal experiment, that is, the effect of administering alkali to dogs that had previously received a lethal dose of curare.

Dogs received, intravenously, 5.4 mg. of curare per kilo body weight. Eight or ten minutes after injection, when paralysis of the muscles, except the respiratory muscles, had appeared, a 5 per cent solution of $NaHCO_3$ was injected intravenously. Complete recovery occurred within fifteen minutes after approximately 3 grams of $NaHCO_3$ had been introduced. The 'curare effect' did not reappear.

At the onset of muscle paralysis the pH and $CO_2$ capacity begin to fall. The acidosis produced at this stage evidently augments the effect of curare, but is not, in itself, sufficiently pronounced to produce coma. Introduction of $NaHCO_3$ not only corrects anoxemia, but also possibly neutralizes the curare effect at the myoneural junction. All controls died of respiratory paralysis within twenty minutes after receiving curare.

37. *The effect of changes in hydrogen-ion concentration of the blood upon reflex time.* W. F. WENNER AND A. B. TAYLOR, State University of Iowa. (Introduced by W. W. Swingle.) (5 min.)

Previous studies on the effect of changes in acid-base equilibrium on the physiological action of strychnine and curare indicated that an increase in hydrogen or hydroxyl ions might affect neural or neuromuscular factors and thus alter the rate of conduction of the nerve impulse.

Dogs were used as experimental animals. The patellar-tendon reflex time of these normal unanaesthetized dogs varied from 0.00775 to 0.0094 second, with an average of 0.0082 second. The pH of the blood of these normal dogs at the time the records were obtained was about 7.36 and the $CO_2$ capacity 45 volumes per cent. With a drop in pH and $CO_2$ capacity, the reflex time was decreased to about 0.0043 second. An increase in pH and $CO_2$ capacity also brought about a decrease in reflex time. The average reflex time following alkali administration was 0.0057 second. One dog included here showed an insignificant increase of 0.0006 second in reflex conduction rate at pH 7.53 and $CO_2$ capacity 72.97 volumes per cent.

The records obtained show clearly that the reflex time of dogs is markedly reduced following acid, and to a less degree following alkali, administration. It is suggested that an increase in H ions probably blocks out the higher centers ordinarily involved in the arc. On the other hand, OH ions probably aid in facilitating the conduction of the nerve impulse.

38. *A study of the rate of oxygen consumption of different Cladocera clones derived from a single mother.* V. OBRESHKOVE AND A. M. BANTA, Saint Stephen's College, Columbia University, and the Department of Genetics of the Carnegie Institution of Washington. (15 min.)

In 1917, in one of the strains of Simocephalus exspinosus, of the many Cladocera reared parthenogenetically in the laboratory of Dr. A. M. Banta, there appeared morphological mutations exhibiting sex intergradedness. Two lines were isolated, and these were bred separately as a separate individual parthenogenetic pedigree. Although at the time this study was made the sex-intergrade character was only slightly manifested, differences in reproductive vigor were still sufficiently in evidence. Employing Thunberg's microrespirometer as modified by Fenn ('27) to detect very minute quantities of oxygen consumption, a study was undertaken of the rate of oxygen consumption of these two clones derived from a single mother and bred for a long time only parthenogenetically. The rate of oxygen consumption was compared with that of the individuals in the 'normal' stock derived from the same mother from which the clones showing mutations were obtained.

The individuals in the normal stock showed a rate of oxygen consumption almost twice that of each one of the other clones, at the same age and size considered. The rate of oxygen consumption of the normal stock was found to be $794 \times 10^{-6}$ cc. per hour per individual. The rate of one of the formerly strongly intergrade stock was $452 \times 10^{-6}$ cc. per hour per individual. Thus the method employed proved very adequate in the measurement of the physiological states of different clones of animals derived from a single individual at a precise stage of development.

*39. Suggestions as to the true nature of 'respiratory X.'* Edwin B. Powers, University of Tennessee. (Lantern; 15 min.)

"Any sample of blood, and the gases with which it is equilibrated, constitute a physicochemical system." It stands to reason that the pH of the fluid of the respiratory center is not always the same as the pH of the blood, due to the free movement of the $CO_2$ between respiratory center and blood, unless there is a substance equivalent to hemoglobin in the fluid of the respiratory center.

The lagging behind in the specific alkalinity-acidity (i.e., the decrease in alkalinity—hemoglobin by decrease in $O_2$ pressure—and an increase in total amount of oxyhemoglobin—red blood corpuscles) hemoglobin-oxyhemoglobin in proportion to the $HCO_3'$ to $CO_2$ ratio on the alkaline side in acclimatization to low barometric pressure and, vice versa, to high barometric pressure are sufficient to explain overbreathing in one case and depressed breathing in the other.

The above lends itself to a mathematical analysis.

*By demonstration*

*40. Apparent relation between peristomal gland cells and endodermal secretory cells of Palmatohydra oligactis (Pallas).* C. H. McConnell, University of Virginia. (Introduced by Wm. A. Kepner.)

Paramoecia in a 7.8 pH solution when injected into a normal hydra are digested in a short period of time. The endoderm upon being sectioned appears normal. On the other hand, paramoecia, in a 7.8 pH solution when injected into a hydra, lacking peristome, remain alive in some cases as long as five hours. In all of these latter cases the hydra finally ejected the living paramoecia, which swam freely away. Accompanying the egested paramoecia was a conglomeration of cell-like bodies. When the incomplete hydras were sectioned, all the endodermal cells were glutted with zymogen bodies and the coelenteron was found to contain many bodies similar in appearance to those egested along with the living paramoecia. In all cases the hydra had been kept foodless for at least twenty-four hours previous to injection with paramoecia. Eight to twenty-four hours were allowed for regeneration previous to experimentation upon the hydra from which the peristomes had been removed. One hour previous to injection, both types of hydra were washed out with an alkaline solution. It would appear that there is such a specific relation between peristomal gland cells and endodermal secretory cells that the latter cannot function except in the presence of the former.

*41. Charts illustrating the effect of light on the metamorphosis of the larvae of ascidians.* Caswell Grave, Washington University.

*42. The body size and the rate of fission in individuals of the protozoan Colpoda.* Edward F. Adolph, University of Rochester.

The data shown describe the regulation of adult size in Colpoda under the influences of oxygen tension, composition of medium, quality of food, quantity of food, temperature, and other factors.

43. *The effects of gonadectomy in Triturus viridescens.* A. Elizabeth Adams and Elizabeth S. Kirkwood, Mount Holyoke College.

In the fall of 1927, males and females of Triturus viridescens were gonadectomized and in some cases the fat-bodies were removed simultaneously. After varying periods of time, the animals were examined and the presence or absence of gonadal tissue and the condition of the reproductive ducts and secondary sexual characters were noted. Of seventy-two males examined from thirty-three days to a year after castration, sixty-four showed no testicular tissue, while five contained one lobe on either right or left side and three a lobe on each side. The evidence as to whether testicular lobes in these eight cases represent regenerated or unremoved tissue is being examined. In all of the animals lacking testicular tissue and to some extent in the others (with one exception), castration effects were apparent in a reduction of external secondary sexual characters (pads on hind legs and toes, cloacal enlargement, tail fin) and a failure of these characters to reappear at the spring breeding season in animals kept alive beyond that time. Internally, the wolffian ducts and ureters were atrophic. In cases examined histologically, the former were devoid of spermatozoa, while the cells of wolffian ducts, of ureters, and of cloacal glands appeared to have lost their secretory activity. Among nineteen females ovariectomized and examined after fifty-eight to 292 days, only one was found which contained any ovarian tissue (three eggs), and all of the animals possessed atrophied oviducts.

44. *The effects of ovariotomy in the brown Leghorn fowl.* L. V. Domm, University of Chicago.

A general demonstration of the effects of ovariotomy contrasted with the normal condition in illustration of the paper presented at this meeting by the writer. To include histological preparations of the rudimentary right gonad of the normal female contrasted with its later development in the ovariotomized female. Preparations of the sacrum with urinogenital system intact showing the types of gonad developed and their relation to the genital ducts in normal and experimental birds. Preparations of skin and feather charts showing types of feathers developed. Photographs of various sexual types; also of certain preparations.

45. *Demonstration of the positive action of the placental hormone on the oviduct of fowls.* Mary Juhn, University of Chicago, and R. G. Gustavson, University of Denver. (Introduced by F. R. Lillie.)

46. *A rapid quantitative precipitin technique.* Alan Boyden and Joseph G. Baier, Jr., Rutgers University. (Introduced by Thurlow C. Nelson.)

The volumes of precipitate obtained from the interaction of antiserum and antigen are measured in Van Allen thrombocytocrits. Definite amounts of antigen and antiserum are measured into clean glass tubes, mixed, and incubated for one hour at 37.5°C. The contents of the tubes (tests being made in duplicate) are then drawn up by suction into the thrombocytocrits and centrifuged for two fifteen-minute periods, at 2150 to 2200 R.P.M., the volumes of precipitate being recorded at the end of each period. The thrombocytocrits are then emptied and washed by drawing up in succession and shaking well, saline, distilled water, alcohol, and ether. They are then dried over a lamp. Series of repeated tests have shown a remarkably small error when groups of four readings are averaged. The technique proposed seems simpler and more rapid than any other quantitative precipitin technique and can be used for a variety of purposes, including the measurement of biological relationships.

*46 a. The effect of $CO_2$ on the respiration of the duck.* THEODORE KOPPÁNYI, Syracuse University.

*By title*

*47. The pedal rhythm of the rock barnacle.* WILLIAM H. COLE, Rutgers University.

In Balanus balanoides L., at Mount Desert Island, the cirri execute rhythmic movements concerned with external respiration and feeding. The regularity and rate of the movements are functions of environmental conditions, and of internal factors. A study of some of the former has revealed the following facts: 1) in quiet water movement becomes irregular or ceases, even when food is present; 2) in running water movement continues regular at a constant rate when other conditions are constant; 3) under constant conditions there is an optimum rate of flow which depends upon the relation between the mass of individuals and the volume of the container; 4) running water is not a mechanical stimulus, since water repeatedly used causes irregularity or closure of the valves; 5) the pH range is from 7.8 to 8.6 (optimum 8.0 to 8.2), within which irregularity of movement is rare; 6) water saturated with carbon dioxide or water heated to 80°C. and then cooled causes irregularity; 7) aeration of water repeatedly used until closure occurs does not restore regularity of movement; 8) progressively diluted sea-water containing more than 3 per cent fresh water produces slower rates of movement, irregularity, and finally closure (10 per cent). This effect is not due to changes in pH; 9) at equal molecular concentrations the following substances are effective in causing irregularity and closure as indicated: chloretone>caffeine>chloral hydrate>magnesium sulphate; 10) the rhythm is not altered by varying the illumination of the animals from darkness to that produced by a 200-watt Mazda lamp at 150-mm. distance. Temperature effects on the rhythm will be reported elsewhere.

*48. Behavior and reactions of a larval trematode, especially to changes of light intensity.* HARRY M. MILLER, JR., Washington University.

Cercaria cita (sp.nov.) is an intermittent swimmer. A sudden and maintained decrease in the intensity of light causes a swimming, light-adapted cercaria to stop, temporarily, after approximately two seconds. In many cases when the duration of reduced illumination was shortened to less than this normal reaction time, there was an inhibition of swimming almost simultaneous with the succeeding abrupt increase of light intensity; but often there was no inhibition, especially when the period of reduced illumination was much less than two seconds. Similarly, the swimming movements of a dark-adapted cercaria are inhibited, after approximately two seconds, by a sudden and maintained increase of light intensity. In the response to either decreased or increased intensity, the reaction time is independent of the intensity of light between about 0.5 and 13,000 meter candles.

The same stimuli may cause individuals resting on the bottom of the container to swim; these reactions are not so easily obtained as those in which swimming larvae are inhibited. Neither stimulus activates passively sinking individuals. A photokinetic effect of light on Cercaria cita was observed in several experiments. This larva is strongly photopositive; various attempts to make individuals photonegative were not uniformly successful. It is not stimulated to swim by mechanical shock, as are the cercariae of many other trematodes.

### 49. *Relation between rate of locomotion and intensity in the beetle Dineutes americanus.* Leonard B. Clark, Johns Hopkins University. (Introduced by S. O. Mast.)

The rate of locomotion of the whirligig beetle, Dineutes, was found in different intensities from 0.02 to 8000 m.c. Apparatus and methods were devised such that during each test the organism remained in a constant intensity and that successive tests could be performed at different intensities without handling the animals.

The evidence secured shows the following: In dark-adapted insects exposed in constant illumination of 4000 m.c., the rate of locomotion is at first relatively high, but it gradually decreases until, at the end of fifteen minutes, the rate becomes constant. In an intensity of 8000 m.c., the rate is at first low, but it increases as the time of exposure increases until, at the end of about twenty minutes, it becomes constant and remains so for two hours or more. This constant rate is the same as the rate in 4000 m.c. after fifteen minutes' exposure. In dark-adapted specimens the rate of locomotion increases as the intensity from 0.02 to 0.5 m.c., then it remains constant until an intensity of nearly 8000 m.c. is reached, after which it decreases. In specimens exposed to the illumination to which they are adapted, the rate of locomotion is always the same for all intensities above 0.5 m.c.

It is concluded that the rate of locomotion depends upon the intensity in high and low, but not in medium illuminations and that the condition of adaptation of the eyes is an important factor in the rate of locomotion in all intensities.

### 50. *Artificial insemination of female guinea-pigs as a method of investigating problems in the physiology of mammalian spermatozoa.* William C. Young, University of Chicago and Brown University. (Introduced by Carl R. Moore.)

The artificial insemination of female guinea-pigs with sperm removed from the epididymis of the male has been found to be a practical technique in the investigation of the action of high temperature on the reproductive capacity of guinea-pig sperm, and in an investigation of changes undergone by sperm during their passage through the epididymis.

If the epididymides of normal adult guinea-pigs are macerated in approximately 10 cc. of Locke's solution at either body temperature or room temperature, and 0.5 to 1.0 cc. of the sperm suspension introduced into the body of the uterus of females during oestrum by means of an especially made catheter or ordinary medicine dropper, 65 to 75 per cent fertile inseminations can be obtained. It is advisable to hold the female ventral side upward and quietly for five minutes following the insemination.

As many as six females, of which four became pregnant, have been inseminated with sperm from one male in the course of a period extending over thirty to forty-five minutes. The number of inseminations which can be made at one time is usually limited by the number of females in heat, although there is a rapid loss of fertilizing capacity by the sperm during the procedure.

### 51. *Methods of inducing the production and the hatching of sexual eggs of Daphnia longispina.* THELMA R. WOOD, Carnegie Institution of Washington. (Introduced by Arthur M. Banta.)

In a study of the inheritance of characters in sexual reproduction, it was necessary to attempt to secure sexual eggs and young in numbers. In the course of this work the following observations were made. By moderately crowding the usually parthenogenetic Daphnia longispina females, sexual eggs and the males necessary for their fertilization were produced. Large numbers must be cultured to insure obtaining both of these simultaneously. As is well known, hatching of these eggs is difficult. Among methods tried were drying and keeping dry for varying periods, changing specific gravity of the water, aeration, removal from the egg case, treatment in light and dark, storing dry outdoors in winter and indoors at laboratory temperatures, and storage for varying periods in constant-temperature cabinets (5° to 20°C.) at the Boyce Thompson Institute—some being stored wet and some dry. In all, during a period of three years, some 6100 eggs were obtained from genetically comparable material. Nine hundred and thirty-seven eggs were hatched. Most variations of the treatments proved ineffective. Greatest success was obtained by storing eggs dry at laboratory temperatures for about fifty days and then (in diffuse daylight) adding dilute filtered culture medium, which was renewed about every ten days. Under this treatment, 40 per cent of the eggs of the last series hatched. Viability of eggs stored dry longer than five months was decidedly reduced, but there was no reduction in viability of eggs stored dry about fifty days and then stored in water five or six months longer.

### 52. *Regeneration in Daphnia magna.* B. G. ANDERSON AND L. A. BROWN, State University of Iowa. (Introduced by W. W. Swingle.)

Experiments designed to determine the relation between the rate of regeneration and age in Daphnia magna have shown no differences in the adult instars (one to eleven). The method followed was to crush with a ball-pointed needle a small area of the carapace, working from the inner side in the early part of the instar before the one on which observations were made. The wound area thus secured in most cases approximated a circle. The perimeters of the wound area were, on the average, no more than 7 per cent greater than that of circles with equal areas. Since regeneration occurs with variation in two dimensions, the third being constant, the amount can be determined by measurement of the areas. Measurements were taken on 305 animals.

The data so acquired lent themselves readily to the study of the amount of regeneration in respect to size of the wound. The following parabolic equation expresses the relation between y, the area of regeneration, and x, the total amount of regeneration possible (wound area), where the time and the temperature are constant, $y = \sqrt{k(x+p)} - h$, where k, p, and h are constants referred to multiples of p, the focal distance, k is 4p and h is 2p.

### 53. *Histolytic influence of atrophying gills of anurans during metamorphosis, with special reference to resistance of fore-limb integument.* E. R. VAN DER JAGT, State University of Iowa. (Introduced by O. M. Helff.)

A previous paper by Helff has shown that the atrophying gills of anurans are responsible for the formation of the opercular fore-limb perforation during metamorphosis. The present work is an attempt to determine the stage of gill atrophy most active in producing such integumentary histolysis.

Homoplastic transplants of gills in various stages of atrophy were made beneath back skin of metamorphosing Rana pipiens tadpoles and speed of integumentary histolysis recorded. The results indicated that histolytic influence of the atrophying gills is accumulative. It is very weak during early stages of gill atrophy, but increases proportionately with increasing atrophy, reaching a maximum just prior to the release of the fore limbs. Following this stage, the histolytic influence of the gills gradually subsides as the gills undergo their final stages of atrophy.

Operations were also designed to throw light on the apparent resistance of fore-limb integument to histolysis. These consisted in placing atrophying gill tissue in contact with the epidermal and inner surface of fore-limb integument grafts. When in contact with the inner surface, rapid histolysis was produced. Contact with the epidermal surface, however, produced little, if any, histolysis. The results indicate that failure of fore-limb integument to histolyze during metamorphosis when in contact with the atrophying gills is due to the fact that the latter are in contact only with the epidermal surface of the skin. The heightened metabolism of the rapidly developing integument also probably tends to resist histolysis.

54. *Integumentary pigmentation in the frog (R. pipiens), during metamorphosis, with especial reference to tail-skin histolysis.* V. F. LINDEMAN, State University of Iowa. (Introduced by O. M. Helff.)

Several types of larval skin-transplantations experiments were made in an attempt to determine the local specificity of frog integument during metamorphosis when transplanted to foreign regions. The grafts involved skin from the back, belly, side, and tail. Observations were made with respect to the specificity of integument for pigmentation and histolysis.

Autoplastic and homoplastic larval skin grafts showed local specificity for pigmentation when allowed to metamorphose. All grafts invariably developed the pigment pattern characteristic of the region from which they had been removed. It is concluded that the integument of the frog (R. pipiens) is specific for pigmentation and the factor responsible for the development of the normal pattern is contained within the skin itself.

Similar transplants, involving an interchange of tail skin with skin from the back, suggested the specificity of tail skin toward histolysis. Tail skin when transplanted to the back undergoes histolysis during metamorphosis. Conversely, skin transplanted from the back to the tail undergoes normal development during metamorphosis and develops the pigmentary pattern characteristic of the region from which it was taken. It is concluded that two factors determine the histolysis of tail integument: one which lies within the skin itself and is specific, and the second, within the blood stream, which becomes functional in inducing histolysis of tail integument at a certain stage of metamorphosis.

55. *Tail atrophy of anuran larvae (R. pipiens) during metamorphosis, with respect to the growth of the urostyle.* O. M. HELFF, State University of Iowa.

The investigations of Bataillon, Mercier, and W. Morse have tended toward the assumption that the atrophy of the tail of anuran larvae during metamorphosis is due primarily to the growth of the urostyle. This rapidly developing organ, by interfering with the blood supply, was thought to result finally in acidosis of the musculature of the tail with resultant autolysis of that organ.

The urostyle of the adult develops from a small cartilaginous rod 2 to 3 mm. in length. This anlage, located near the junction of body and tail and deeply embedded in close proximity to several blood vessels, is difficult to extirpate. Operations made from the dorsal or ventral sides were unsuccessful. A lateral approach finally yielded the desired results. Twelve successful cases were obtained of urostyle-anlage extirpation in normal tadpoles. The larvae were then allowed to metamorphose. The tails of these individuals underwent normal atrophy, during metamorphosis, identical in appearance and time with the tail atrophy of normal unoperated individuals. Subsequent autopsy revealed no regeneration of urostyle cartilage.

The conclusion is drawn that the developing urostyle of metamorphosing anurans has no bearing or causative influence on the atrophy of the larval tail. Present results suggest that the tissues of the tail are specific in their ability to undergo degeneration at a certain metamorphic stage. It is quite probable that the stimulus for such histolysis is transported through the blood stream and is probably hormonic in nature.

*56. Effect of prenatal and postnatal injections of the pituitary gland in the white rat.* E. L. COREY, Yale University and Cold Spring Harbor Biological Laboratory. (Introduced by H. W. Stunkard.)

A series of experiments was conducted in an attempt to ascertain the earliest activity of the pituitary hormone. Fresh, macerated pituitary glands of the white rat were injected pre- and postnatally into young rats, and the effect of such injections upon the gonad was ascertained by histological examination. The precocious differentiation of the gonad was assumed to be indicative of tissue response to the hormone.

In an earlier study (previously reported), normal saline solution was used as an injection medium. Later, fresh blood plasma from the albino rat was employed, in the hope of producing more precocious gonad differentiation than that recorded when using the normal saline solution. One hundred and fifty-six animals, comprising twenty-one litters, were subject to injection. Six litters furnished the controls.

Prenatal injection in no way hastened the differentiation in the gonad, indicating that the gonad of the rat foetus is incapable of response to the pituitary hormone. The earliest advance in maturity of the injected animals over the controls appears, in both series, between the tenth and fifteen days of postnatal life. The effect is marked in both sexes. The use of blood plasma as a medium for injection did not hasten the differentiation of the gonad to any greater degree than did normal saline solution.

*57. The effects of daily injections of bovine anterior-pituitary extract upon the developing albino rat.* GEORGE EDWIN JOHNSON AND EVERETT DUANE SAYLES, Kansas State Agricultural College.

Daily injections of bovine anterior-pituitary extract in both male and female rats from the fifteenth to the one hundred and fiftieth day of life resulted in an acceleration in growth over that of saline-injected litter-mate control rats of the same sex. The average weight of seven experimental male rats became greater than that of seven litter-mate control males at thirty-five days of age. In the same number of females and their controls this point was reached at forty-five days of age. At 150 days of age, all of the treated rats of each sex were heavier than their controls, the females very significantly so, but the males not quite significantly so. The leg bones averaged slightly longer, but not significantly so in the extract-injected animals.

The extract produced an enlargement of the ovaries of the females, due to the development of a large number of corpora lutea. Several corpora lutea and follicles contained degenerating ova. Other reproductive organs of either sex did not appear to be affected by the extract.

58. *The effect of mammalian follicular extract on the oviducts of the frog (Rana pipiens Shreber).* OPAL M. WOLF, University of Wisconsin. (Introduced by Frederick L. Hisaw.)

A study of the relationship of the ovaries to the hypertrophy which occurs normally in the müllerian ducts of the frogs during the breeding season has been undertaken. Ovariotomies were performed, so that the condition of these structures in castrated animals could be compared with those of normal females. When female frogs were castrated in September, a marked degeneration occurred as early as December. The glandular cells in the oviduct were small, the cytoplasm appeared vacuolated, and very few secretory granules were present. The involution of these structures after castration appears to be a gradual process, because the oviducts of females castrated in December and killed in April did not show so striking a degree of degeneration.

Daily injections of mammalian follicular hormone beginning with ½ rat unit and increasing the amount to 2 rat units were given daily for a period of fifty-five days. When the animals were killed, April 18th, it was found that the injections had prevented the atrophy of the oviducts and had maintained a normal and in some cases hypertrophic growth. The lack of ovarian influence rather than an inhibitory effect of the testicular secretion is suggested as the causative factor which brings about the rudimentary condition of the oviducts in the male, because no increase in size was noted in these structures nine months or more following the removal of the testes.

59. *Demonstration of the positive action of the placental hormone on the oviduct of fowls.* MARY JUHN, University of Chicago, AND R. G. GUSTAVSON, University of Denver. (Introduced by F. R. Lillie.)

The effect of the placental hormone prepared by one of us (R. G. G.) was tested this year on seven immature pullets, six sister birds serving as controls. Each test bird was given daily subcutaneous injections of 2 cc. over a period of ten days of a placental preparation assayed to contain 15 rat units per cubic centimeter. Each pullet received a total of 300 rat units. The controls were run simultaneously on 2 cc. of olive oil, this serving as suspension medium for the hormone. The birds were autopsied at the end of the experiment, and each test oviduct was found to be roughly ten times the weight of that of any one of the controls. Accompanying this increase in weight there was a histological development of the glands of the oviduct comparable to the condition found in the adult hen, whereas the development of the glands had not yet begun in the controls.

Previous injections carried out in the same manner, but with a lower dosage, 4 rat units daily to a total of 40 rat units per bird, gave negative results. Preparations from the egg yolk, negative when tested in mammals, were also so in birds.

The response of the immature oviduct of the fowl to placental preparations is uniform when a sufficiently high dosage is established. This pronounced lack of taxonomic specificity might be interpreted as suggesting a relatively simple chemical nature of the hormone. Riddle's fluctuating findings in the oviduct of pigeons may be due both to insufficient concentration and length of time over which his injections were extended.

### 60. *The lack of specific effects of endocrine substances upon Cladocera.* L. A. BROWN, State University of Iowa, AND ARTHUR M. BANTA, Carnegie Institution of Washington.

The products of the endocrine glands, being primarily of vertebrate origin, are known to have specific effects on the growth and differentiation of vertebrates, but their action on invertebrates is still in dispute. According to recent writers, thyroid products do not accelerate fission rate in the Infusoria. The effects of thyroid and other endocrine products on the attainment of maturity in arthropods are not so clearly understood.

In the course of certain experimental work on sex control in Cladocera, it was found (as Van Herwerden had previously reported) that filtrates of desiccated adrenal cortex (ox) produced a marked acceleration in rate of development of these animals, as indicated by the time of release of their first parthenogenetic young. Experiments were employed to test this apparently specific nature of the adrenal cortex. In the first of these filtrates of other endocrine substances, adrenal medulla, thyroid, thymus, etc., were used. All of these gave the same qualitative and quantitative result as the adrenal cortex, namely, a shortening of the time to the release of the first clutch of young, as compared with sister animals not so treated. A still more conclusive proof of the lack of the specificity of these effects was obtained through the parallel use of a filtrate of dried muscle tissue (ox). The reduction in time to the release of the first young was strictly comparable to that found with the endocrine products.

The results obtained are probably due to changes in the bacterial flora which serve as food for the cladocerans.

### 61. *The effects of adrenalectomy in the lizard.* A. ELIZABETH ADAMS AND MARGARET HARLAND, Mount Holyoke College.

Adrenalectomy was performed on male lizards (Anolis carolinensis) in an attempt to discover whether the adrenals are essential to life in this form, whether compensatory hypertrophy occurs after unilateral extirpation, and whether adrenal removal affects the gonads. Unilateral removal was done upon eighteen animals, thirteen of which were later used for bilateral extirpation.

Removal of one adrenal seemed to have no effect upon the general condition of the animal, providing that recovery from the operation was complete. Compensatory hypertrophy of the remaining gland examined after varying periods of time (ten to 104 days) following unilateral adrenalectomy was not observed, except possibly in one case. In this instance the cortical tissue appeared to be somewhat in excess of that in the normal gland removed fourteen days previously. However, a wide variation in size of the normal glands, even of the two glands of one individual, and insufficient material make it impossible to state that compensatory hypertrophy did not occur. Unilateral removal apparently had no effect upon the testes.

Excision of the remaining gland ten to 104 days after extirpation of the other proved fatal in all cases except one. The period of survival ranged from 8 to 144 hours, two animals living 8, five 24, two 48, one 72, one 96, one 144 hours. (The thirteenth animal was killed after 144 hours.) The condition of these animals shortly before death was characterized by loss of appetite, weakness, paralysis of the hind limbs, and, finally, loss of movement.

*62. Studies on amphibian neoteny. 1. Metamorphosis of the Colorado axolotl by the injection of inorganic iodine.* W. R. Ingram, State University of Iowa. (Introduced by W. W. Swingle.)

Experiments with the Colorado axolotl, to be properly controlled, must be carried out with hypophysectomized and thyroidectomized individuals, since this variety of axolotl metamorphoses spontaneously when removed from its native habitat. Individuals submitted to such treatment have been considered never to metamorphose, except under the influence of a glandular replacement therapy or upon the administration of organic iodine compounds. However, following the subcutaneous implantation of small crystals of inorganic iodine, hypophysectomized and thyroidectomized axolotls rapidly passed through the transformation stages to the adult condition. This confirms the observations of Blacher and Belkin upon the European axolotl—a form which never metamorphoses spontaneously. Most investigators have considered inorganic iodine to have negligible influence upon the metamorphosis of urodeles. It is now apparent, however, that large amounts of inorganic iodine administered subcutaneously or intraperitoneally do markedly accelerate differentiation in neotenic forms.

*63. The transplantation of ovary into capons.* L. V. Domm, University of Chicago.

A series of subcutaneous and intraperitoneal ovarian grafts were made into capons and cockerels. Twenty-seven young capons and nineteen cockerels received a total of 165 grafts. Owing to the great resistance of the host, only three capons were successful.

The successful cases, still living, have all developed some female plumage, though none completely. No. 507 developed female feathers at four months following the initial implantation. New feathers observed during the following six months were female. Following this, the new plumage became intermediate in type and has remained so. Hence, except for sporadic male feathers, found principally in the tail, the bird has an intermediate plumage. No. 508 likewise developed female feathers at four months after the initial implantation. New feathers during the following twenty-one months were female, while during the past two months they have been intermediate in certain areas and male in others. No. 547 developed female feathers within five weeks after the initial implantation and has continued to develop such plumage during the following thirteen months to the time of writing. All three capons have developed prominent head furnishings approximating those of the male in size, but a loppy comb like that of the female. Spurs are well developed. No. 508 crows and attempts treading. No. 547 crows and treads.

These results contrast with the earlier observations of practically complete feminization in such cases and give rise to the suspicion that medullary, as well as cortical, elements have become activated in the growing graft.

### 64. *Transplantations of compensatory right gonad and normal rudimentary right gonad of the female brown Leghorn into cocks and capons.* L. V. Domm, University of Chicago.

As previously shown (Domm, '27), the female fowl, following ovariotomy, gradually develops the typical secondary sex characters of the male, but ultimately reverts to female plumage, while the other male characters are retained. Examination of the gonad sites revealed a testis-like organ on the right which, when removed, caused the reassumption of male plumage and other capon characters. In a series of experiments in 1925 and 1926 the right compensatory gonad, and the normal rudimentary right gonad were grafted subcutaneously and intraperitoneally in cocks and capons. None was successful.

During the summer of 1927 this problem was again attacked. Fourteen young capons were selected, and into each were grafted subcutaneously parts of right compensatory gonads. They received from two to four grafts each—a total of thirty-seven. None was successful. After a period of four months, five of these capons were selected and given additional subcutaneous grafts consisting of entire right compensatory gonads from recently ovariotomized birds. These five capons received from six to fourteen grafts each—a total of forty-one. None of these became successfully established. Why such engrafted gonads should fail to grow in the tissues of the host is problematic, though certain reasons suggest themselves. Our experience with autoplastic and homoplastic grafts in male fowls has proved our technique adequate. It is felt that lack of relationship between donor and host and the firm consistency of the engrafted gonads which consequently do not lend themselves readily to vascularization are factors involved.

### 65. *The effect of gamma radiation on the permeability of frog's skin to NaCl.* Marvin M. D. Williams, University of Pennsylvania and Philadelphia General Hospital. (Introduced by J. H. Bodine.)

The purpose of these experiments was to study the effect of gamma radiation on the permeability of a membrane to a salt. After removing the skin from a frog, half of the skin from the ventral and half from the dorsal surfaces were exposed to the gamma rays from radium emanation. Sections from both the radiated and non-radiated portions of the skin were then used to separate a 0.65 per cent solution of NaCl from distilled water. The amount of NaCl which diffused through the skin into the water was determined at frequent intervals until the skins broke down. For a period of ten to fifteen hours after radiation there was no difference between the rate of diffusion of the NaCl through the radiated and non-radiated portions of the skin. After this latent period, the rate of diffusion through the radiated portions was less than through the non-radiated portions. The radiated portions did not break down as quickly as the non-radiated portions.

### 66. *The effects of radium radiation on the oxygen consumption of grasshopper eggs.* Reuben Blumenthal and Marvin M. D. Williams, University of Pennsylvania and Philadelphia General Hospital. (Introduced by J. H. Bodine.)

In almost all experiments carried out to determine the effects of x-rays and radium radiations, the material used has been of such a nature as to permit of determinations of end points alone. Greater importance, however, lies in the rate at which affected or resulting reactions proceed, and the present study was begun in order that some such knowledge might be gained. The oxygen consumption of developing eggs of Melanoplus differentialis has been measured. Eggs were exposed to definite strengths of radium emanation at various distances and for definite periods of time. The oxygen consumption was then measured by means of a modified Krogh's differential manometer. It was found that weak doses of radium produced a marked increase in oxygen consumption, while strong doses produced a decrease. The stimulating effect also manifested itself in a hastening of the hatching time, while the inhibiting effect showed itself in a retardation of the hatching time. Immediately after exposure of the eggs, a latent period is evident, extending over a period of about 120 hours, during which time the oxygen consumption remains approximately the same as in the normal eggs. Increasing the distance between the eggs and the radium tubes decreased the effects of the retarding doses and increased the effects of the stimulating doses.

### 67. *The specific conductivity of protozoan cultures.* Elmer C. Herber, Eleanor Slifer, Reuben Blumenthal, T. P. Sun, C. C. Wang, University of Pennsylvania. (Introduced by J. H. Bodine.)

The variety of methods used in making up protozoan cultures suggested the comparison of some physicochemical properties of such cultures. The present study deals with the specific conductivity of protozoan cultures. The cultures were made up with boiled hay in boiled pond water, dry hay in boiled pond water, and a culture containing merely boiled pond water. All cultures were inoculated from a single stock of paramecium. Conductivity readings were taken daily from the beginning of February until the end of May. The results show that the cultures consisting only of boiled pond water had the lowest specific conductivity, which value remained remarkably constant throughout. The hay-boiled water cultures had a somewhat higher specific conductivity and the degree of fluctuation was more marked. The dry hay-boiled water cultures had the highest specific conductivity, and here the amount of fluctuation was greatest. Although each culture varied one from the other in its degree of specific conductivity, all the cultures maintained their relative positions throughout the length of the experiment. Temperature effects were evident only when a sudden and sharp drop or rise occurred. There are indications that a rhythm of conductivity occurs in the cultures.

*68. Recovery of the heart beat of Fundulus embryos after stoppage by potassium chloride.* Melvin W. Thorner, University of Pennsylvania. (Introduced by J. H. Bodine.)

Experiments were conducted following those of Loeb and Cattell ('15) on the effect of various factors influencing the recovery of heart beat in Fundulus heteroclitus embryos after anaesthetization by KCl. It was found that the time of recovery is directly proportional to the [KCl] and to the time of immersion in the KCl solution when the recovery is effected in sea-water. It was also found that the hearts of anaesthetized embryos may recover in distilled water after sixteen hours' immersion in M/2 KCl, the mortality being no greater than 25 per cent. The relations between anaesthetic doses at various concentrations and the corresponding period of recovery in sea-water were noted. The recovery in distilled water was studied with the aid of electrical conductivity apparatus. Results obtained indicate that KCl diffuses out of the egg, thus effecting recovery. The effects of various electrolytes which are components of sea-water were observed when such salts were used as recovery solutions.

*69. The hydrogen-ion concentration of the contents of the alimentary tract of albino rats.* Reuben Blumenthal, Elmer C. Herber, Eleanor Slifer, T. P. Sun, C. C. Wang, University of Pennsylvania. (Introduced by J. H. Bodine.)

The universal use of albino rats for experimental purposes emphasizes the importance of the knowledge of the various reactions of the normal rat. The hydrogen-ion concentration and the protozoan number of the alimentary tract were chosen for the present detailed study. The portions studied were the stomach, small and large intestines, and caecum. pH determinations were made electrometrically, and one hundred rats, of both sexes and varying in age from fifteen days to two years, were used. It was found that the small intestines had the most constant pH, while the greatest fluctuations occurred in the stomach. Average figures for both male and female, with their ranges, follow: stomach, pH 3.9, range 3.1 to 4.6; small intestines, pH 7.0, range 6.6 to 7.4; large intestines, pH 6.4, range 6.0 to 7.1; caecum, pH 6.5, range 5.4 to 6.9. Very slight differences occurred with variations of age, body weight, and body length. Sixty-day rats had the largest protozoan numbers, in the small intestines and caecum, whereas the fifteen-day rats, which were milk-fed, contained no protozoa whatever.

*70. Neutral red and salt antagonism in the Fundulus egg.* J. H. Bodine, University of Pennsylvania.

Experiments were conducted to test the validity of the supposed similarity between salt antagonism and neutral-red salt antagonism, as pointed out by Loeb for the egg of Fundulus heteroclitus. By using eggs with and without the chorion, it can be definitely shown that neutral red is almost entirely taken up and held by the chorion alone. Results similar to those of Loeb with intact eggs can be obtained with chorions alone where concentrations of dye, electrolytes, etc., are similar on both sides of the membrane. The antagonistic actions of salts on the dye are not necessarily surface ones. The action of intra- and extracellular salts on the staining and destaining reactions of the eggs have been noted.

Intensity of staining with varying pH values and variations in salt content have been investigated and the relative importance of each observed. The approximate iso-electric point of the chorion has been determined and reactions of the egg to neutral red seem best explained upon phenomena connected with such values.

### 71. *The rate of killing of Daphnia magna with varying concentrations of mercuric chloride.* John Breukelman and L. A. Brown, State University of Iowa. (Introduced by W. W. Swingle.)

Adult parthenogenetic females of Daphnia magna were killed by placing them in solutions of mercuric chloride of concentrations from 0.1N to 0.0001N. The experiments were all conducted at room temperature (21°), and the animals were all from the same clone, having been reared under uniform cultural conditions for many generations.

Killing curves were obtained by getting the per cent of the animals of a given lot that died after various time intervals in the poison. These results were checked by direct microscopical examination of the animals, noting with a stop-watch the time of death, taken as the complete relaxation of the post-abdomen. The sequence of events in the death process was quite constant, and this relaxation was the last observable body movement.

When the percentages dead were plotted against the time intervals, an asymmetrical sigmoid curve was obtained, the point of inflection always being in the first half of the curve. When the logarithms of the reciprocals of the times necessary to kill 50 per cent were plotted against the logarithms of the reciprocals of the concentrations of the toxic agent, a linear relation was obtained, with a break at 0.001N, the greater slope being below this concentration. Campbell ('25–'26) gives a similar relationship for the toxic effect of arsenic in the silkworm when using logarithms of the dosage. The lines for 30 per cent and 70 per cent dead were plotted and found to be parallel to that for 50 per cent.

### 72. *Factors influencing water content and rate of metabolism in developing insect eggs (Orthoptera).* J. H. Bodine, University of Pennsylvania.

Extensive quantitative studies on the changes in water relations and rates of oxygen intake and carbon-dioxide output have been made on eggs of nine different species of grasshoppers. The amounts of water contained in an egg are more or less specific and seem to be correlated with the future history of the egg in respect to its temperature relations and time required for development. Eggs not normally subjected to low temperatures always have the highest amounts of water and undergo but small changes in these amounts during the course of development. Eggs normally subjected to low temperatures have lower water contents and are also able to adjust these amounts, depending upon external temperatures, etc. Water relations in these eggs can be experimentally controlled and regulated.

The rates and total amounts of oxygen and carbon-dioxide exchange during the entire course of development have been determined. Particular attention has been given to rhythms in rates of gas exchange, the effects of temperature, respiratory quotients, formative periods, growth and cell division, relations between water content and rates of gas exchange, etc.

As a result of these studies, it seems quite possible to get definite information concerning many phases of the physiology of development not easily attacked in eggs of higher forms.

*73. Further study of the surface precipitation reaction.* L. V. HEILBRUNN.

When fluid protoplasm emerges from a cell it forms a discrete droplet. The inability of the watery protoplasm to diffuse through the watery medium outside the cell is due to coagulation or precipitation. This is called the surface precipitation reaction. In an earlier report and more especially in a recent book, details of the reaction as it occurs in the sea-urchin egg are recorded. In this egg it is easier to study the reaction than it is in other types of living substance. Often it is difficult to demonstrate the reaction at all, and in many instances it appears to be lacking. However on close and careful study, with varying techniques, it has been possible to demonstrate a surface precipitation reaction of one sort or another in all of the cells that have been examined. These include different types of cells in animals from six phyla, from Protozoa to Chordata. In every instance calcium plays a part in the reaction. For if calcium is removed from the outer medium, no reaction occurs. The evidence therefore indicates that there is a definite type of protoplasmic reaction which is characteristic of the living substance in many, and perhaps in all of its varied forms.

## GENERAL EVOLUTION

### *By demonstration*

*74. The elimination of the male in the evolution of Cladocera.* ARTHUR M. BANTA, Carnegie Institution of Washington.

The ancestors of the Cladocera presumably were marine. In adaptation to fresh-water habitats (lakes, ponds, and even temporary rain-water ponds) cladocerans have undergone numerous adaptive modifications, among which may be included parthenogenesis and the accompanying decreased frequency of males. Through rapid reproduction in the early spring, parthenogenesis provides the following advantages: 1) successful competition with rival dwellers in their habitats and, 2) in rapidly receding ponds, the early attainment of a large population which insures the timely production of males and the sexual 'winter' eggs. Five general types may be considered in the elimination of the male: 1) Ancestral Cladocera, presumably marine; exclusively sexual reproduction, males occurring in every generation. 2) An intermediate type, living in temporary ponds; the reduction in occurrence of males is apparent, but males and the specialized and highly resistant sexual or 'winter' eggs frequently occur (most Daphnia, Moina). 3) An advanced type, living in generally permanent ponds; males and sexual eggs occur infrequently and apparently may fail to appear throughout a year or several successive years (Simocephalus). 4) A more advanced type, living in larger lakes in mild climates; males and sexual reproduction unknown (lacustrine Daphnia). 5) The most highly specialized type, living in temporary ponds like the second type (and perhaps correlative with the third or fourth type); the male does not occur, but the winter egg (here a pseudosexual egg) is readily produced and, like the sexual egg, serves to carry the species over periods of drought or frost (one Daphnia).

*75. Polymorphism among the castes of termites.* ALFRED EDWARDS EMERSON, University of Pittsburgh.

Termites possess several castes with morphological and functional distinctions. The primary castes arranged in order of phylogenetic origin are: 1) Reproductive individuals; 2) soldiers; 3) workers. Each of these primary castes has evolved other more specialized types, however, many of which may occur in the same colony. Sex is only significant in the reproductive castes, the sterile castes being both males and females with degenerate reproductive organs. The more important polymorphic types found throughout the termite series may be listed as follows: 1) First-form winged male, later shedding all except the base of the wing, and becoming the fertile male or king. 2) First-form winged female, later developing into the fertile female or queen. 3) Second-form male or king with wing buds. 4) Second-form female or queen with wing buds. 5) Third-form male or king without wings. 6) Third-form female or queen without wings. 7) Biting mandibulate soldier. 8) Mandibulate soldier with head fitted for blocking passageways. 9) Major mandibulate soldier (Rhinotermes). 10) Minor soldier with specialized labrum and degenerate mouthparts (Rhinotermes). 11) Major mandibulate soldier (Acanthotermes). 12) Medium mandibulate soldier (Acanthotermes). 13) Minor mandibulate soldier (Acanthotermes). 14) Major nasute soldier (Nasutitermes). 15) Medium nasute soldier (Nasutitermes). 16) Minor nasute soldier (Nasutitermes). 17) Snapping mandibulate soldier (Neocapritermes). 18) Major worker (Syntermes). 19) Minor worker (Syntermes).

Examples of these castes, together with intermediate forms and phylogenetic relationships, will be exhibited, the whole demonstration illustrating the various ramifications of the polymorphism of termites better than any collection thus far exhibited.

### 76. *On the occurrence of a fresh-water medusa, Craspedacusta sp., in Stallworth Lake, Tuscaloosa, Alabama.* William E. White, University of Alabama. (Introduced by John Y. Graham.)

*By title*

### 77. *An hypothesis on the origin of graded series of local races in fishes.* Carl L. Hubbs, University of Michigan.

In previous papers I have dealt with environmental correlations in the geographical variation of fishes. Structural differences between local races seem largely the result of changes in developmental rate (and metabolism), not themselves of direct selectional significance. A harmonious relation exists between the developmental rate and the usual environmental conditions during growth. Normal development is possible with some variation in conditions. But when a new territory is populated, with, for instance, a lower temperature, the individuals are brought closer to the threshold of abnormal development and a higher rate of elimination must result. A mutation covering the developmental metabolism would adjust the population to the new habitat. Such changes as an increase in segments would secondarily result. The population would increase and push on into still colder waters, where the same modifications would be expected. A series of such primarily physiological and secondarily structural alterations would produce the graded series of local races often exhibited in the latitudinal variation of fishes.

## PROTOZOÖLOGY

*Read*

78. *Heritability of the effects of chemically different media upon the fission rate within the clone of Paramecium caudatum.* AUSTIN R. MIDDLETON, University of Louisville. (Lantern; 15 min.)

A clone of Paramecium caudatum, consisting of four sets of twenty lines, was cultivated under balanced selection: 1) One set, in Horlick's malted milk prepared with 0.01 per cent $(NH_4)_2C_4H_4O$, during thirty days averaged 72.15 fissions per line, with the range 65 to 81; its control set averaged 83.95 fissions and ranged 77 to 92. For the thirty days following the ex-tartrate lines averaged 55.01 and ranged 47 to 64; their controls averaged 59.03 and ranged 51 to 67. 2) A set in 0.05 per cent $(NH_4)_2SO_4$ for fifteen days averaged 39.15 and ranged 32 to 46; its controls averaged 42.90 and ranged 39 to 49. For the thirty days following, the ex-sulphate lines averaged 55.40 and ranged 47 to 65; their controls averaged 59.30 and ranged 51 to 67. 3) A set in 0.1 per cent $KNO_3$ for forty days averaged 68.45 and ranged 58 to 80; their controls averaged 102.45 and ranged 96 to 113. For the forty days following, the ex-nitrate lines averaged 69.80 and ranged 56 to 79. Their controls averaged 74.55 and ranged 63 to 84.

Transferring from experimental to control medium is somewhat comparable to reversed selection. Especially in the last of these experiments, there has been established a persistent and recognizably different average fission rate between experimental lines and their controls. Here the averages were 68.45 and 69.80, with ranges 58 to 80 and 56 to 79, for the experimental and test periods, respectively. The control average dropped from 102.45 to 74.55.

79. *The biology of sewage disposal—a preliminary study.* H. P. K. AGERSBORG, State of Illinois, Department of Conservation. (8 min.)

The absorption of oxygen from the air pumped into the settled sewage materially aids in the recovery of the dormant organisms there residing, but at the Decatur (Illinois) plant the sewage is so strong, even after it has passed the Imhoff tanks, that a 2.5-hour period of aeration in the aeration tanks is not sufficient to revive life sufficiently that it may live upon, and devour appreciably, the luxuriant growths of Sphaerotilua natans, Zoögloea ramingera, and other bacteria, thereby reducing the load of the sprinkling filter beds. Together with the amorphous matter, they form a flocculent sludge which does not settle out in the settling tanks. This is due to the chemical nature of the sewage. The scavenger organisms which would live upon these do not develop on account of the retarding effect the trade wastes have upon the life-reviving processes in the plant prior to reaching the sprinkling filter beds. Nevertheless, the sprinkling filter beds perform a marvelous piece of work. The influent, devoid of oxygen, becomes sufficiently saturated with it to foster and promote aerobic organisms characteristic of fresh-water ponds. Prolonged aeration promotes the development of chloroflagellates and peritrichous ciliates. Under the best conditions, the life of the river at the inflow of the treated sewage resembles that of the sprinkling filter beds. Schools of fish, including crappies, sunfish, golden shiner—that is, game fish—are fond of the effluent of the sewage-disposal plant when it is mixed with the water of the river.

80. *Relationships of the Opalininae.* Charles A. Kofoid and Mary Louise Dodds, University of California.

Mitosis in two species of the subfamily Opalininae, namely, Opalina obtrigonoidea and Opalina virguloidea, has been studied. At nuclear division an extranuclear centrosome divides and the daughters migrate to the poles of the elongating nucleus, forming an extranuclear paradesmose, as in the flagellates. Each nucleus has a slender rhizoplast running from the centrosome on the periphery of the nuclear membrane to a basal granule—in reality the blepharoplast—from which the flagellum, the so-called cilium, emerges. From this blepharoplast another rhizoplast runs down to the so-called endoplasmic spherules, which are interpreted by us to be parabasal bodies. The unit of the neuromotor system in the species observed thus consists of the following parts: the flagellum, its blepharoplast, parabasal body and its rhizoplast, and, if attached to a nucleus, the rhizoplast running from the blepharoplast to the centrosome on the nuclear membrane. The blepharoplasts are arranged in spiral lines and the parabasal bodies are distributed below them less clearly showing the spiral arrangement. The neuromotor units are thus considerably in excess of the number of the nuclei. A similar relation has been evolved in a number of multicellular types of flagellates found in the termites, in which, as in the genus Calonympha, there are more neuromotor units than there are nuclei. The neuromotor system and the type of mitosis in the opalinid Protozoa are clearly homologous to that of the flagellates. In the light of these facts, it is logical to transfer the Opalininae from the Ciliata to the Flagellata.

81. *The body size and the rate of fission in individuals of the protozoan Colpoda.* Edward F. Adolph, University of Rochester. (Lantern; 12 min.)

The exact times at which individuals and their immediate progeny formed 'division cysts' were ascertained. Feeding upon a clone of flagellates in a constant inorganic medium, Colpoda gave normal variabilities for duration of one generation (coeff. var. 11.1 at 21.6° ± 0.2°, clone A; 9.6 at 19.5° ± 0.1°, clone C). There were significantly shorter durations among those occasional and larger individuals whose parents had thrown only two progeny instead of the usual four. The body volumes of adult individuals whose parents had produced four progeny showed nearly normal variabilities (coeff. var. 19.9 at 21.6°, clone A; 17.8 at 19.5°, clone C); the diameters varied still more nearly normally.

There was zero mass correlation between adult sizes and the durations required to attain these sizes; yet when sister individuals were compared, the smaller individuals had taken less time to grow. Fission rate was therefore a primary factor in the regulation of adult size. But size had little or no influence upon the age at which fission occurred within the range of normal variations. There was zero correlation between the duration of one generation and the duration or the size of the next generation. But smaller sizes conferred shorter lives on the progeny when only two daughters resulted; and individuals of extreme sizes which threw four daughters tended to confer similar sizes upon them.

### *By demonstration*

82. *Microkinematograph of life-history of Paramoecium.* Charles S. Apgar, University of Pittsburgh. (Introduced by R. T. Hance.)

Moving pictures of the activities and reproduction of Paramoecium.

*By title*

### 83. *Modifications of Chilodon uncinatus produced by ultraviolet rays.* MARY STUART MACDOUGALL, Agnes Scott College.

Using a Cooper-Hewitt mercury-vapor quartz lamp, run on 3½ amperes, rapidly dividing and conjugating cultures of Chilodon uncinatus were exposed at varying lengths of time up to two minutes, and at intervals of two or three days, depending upon the state of the cultures. To date several types of animals, able to reproduce without change, have been found:

1. A tetraploid form, the diploid number of eight chromosomes having been checked through four conjugation epidemics. It is like the normal form, except in size and the fact that the number of vacuoles has been doubled.
2. An animal showing both the characteristics of the species uncinatus and cucullulus. This animal has encysted repeatedly and has bred true for over a year.
3. A tailed form which lived forty generations.
4. A giant form still alive after sixty generations.
5. A pair which fused, lived twenty generations. The nuclei fused, and the animal divided as one.

### 84. *Is fission the only method of reproduction in Amoeba proteus?* P. L. JOHNSON, Johns Hopkins University. (Introduced by S. O. Mast.)

Some investigators hold that, by multiple nuclear division, origin of nuclei de novo in the cytoplasm, autogamy, gamete formation, conjugation, sporulation, gemmation, and viviparous reproduction, Amoeba proteus gives rise occasionally to numerous minute amoeboid forms which subsequently develop into adults.

Repeatedly, specimens of Amoeba proteus adapted to diverse environmental conditions were isolated in depression slides and Syracuse watch-glasses. Coincident with their disappearance, numerous minute amoeboid forms frequently appeared—supporting the view above. Various facts obtained, however, oppose this view, namely: 1) These small amoeboid forms never become large amoebae. 2) Sometimes the small amoebae appeared before the disappearance of the large specimens. 3) This obtained frequently, after degeneration of the specimens. 4) Small amoeboid forms appeared on slides without large Amoeba proteus. 5) Under sterile conditions, with or without large amoebae, no small forms occurred, provided the large ones were sufficiently washed. 6) In all tests minute forms found developed either into Mycetozoa or remained small. 7) Weekly observations on 150 Amoeba proteus cultures extending over periods of months revealed no evidence of reproduction other than fission. 8) Small amoebae frequently found in the cultures presented no characteristics like those of the large specimens, namely, type of nucleus, crystals, movement, and pseudopods. 9) Cultures completely depleted of large amoebae never afterward yielded large Amoeba proteus. 10) Parasites and fungi in Amoeba proteus indicate sources of error in interpretations.

These observations indicate that the assertions that reproductive phenomena other than fission occur in Amoeba proteus are due to misinterpretations.

### 85. *The pyrenoid of Chlamydomonas.* J. McA. KATER, Utah Agricultural College.

The pyrenoid of Chlamydomonas nasuta is located along the main axis of the body, just posterior to the nucleus. Thus, it is approximately equidistant from most points on the bag-shaped chloroplast and does not form a part of the latter structure, as it does in most green algae. The pyrenoid consists of a central reticulate core, basophilic to iron-alum-haematoxylin, and a hyaline cortex, divided into sectors by slender strands. When the nucleus is in the early prophase the pyrenoid disappears, not even a vestige of it remaining. This is a gradual process, and can be measured by the loss of affinity for iron-alum-haematoxylin on the part of the central core or by the concomitant enspherulation of the cortical sectors. In the late telophase the pyrenoid is reformed by modification of cytoplasmic alveoli. A single alveolus forms the core, and a number of them contribute to the formation of the cortex. The alveolus which forms the core becomes basophilic to iron-alum-haematoxylin centripetally, and the alveoli which form the cortex become compressed until the entire mass becomes spherical, at the expense of the sphericity of the individual alveoli.

*86. On certain cytoplasmic inclusions in the flagellate, Peranema trichophorum.* Richard P. Hall, University College, New York University.

Two general types of cytoplasmic inclusions, differing in reaction to vital dyes and to osmic fixation and impregnation, have been distinguished in Peranema trichophorum. The mitochondria are small, elongated structures which lie in more or less spiral rows just beneath the periplast. They are stainable vitally with Janus green B, and may be demonstrated by fixation with osmic (Mann-Kopsch; Altmann) and staining with hematoxylin. The mitochondria may occasionally be blackened slightly in osmic impregnation, but are bleached readily with hydrogen peroxide. The other type of inclusions consists of spherical bodies scattered irregularly throughout the cytoplasm. These inclusions are stainable vitally with neutral red, but not with Janus green, and are not demonstrated by osmic fixation followed by hematoxylin. They are blackened consistently in osmic impregnation and are not bleached in the usual treatment with hydrogen peroxide. These spherical inclusions are similar in characteristics to the 'Golgi elements' described in various Sporoza and in metazoan cells.

*87. A study of the metabolism of Euglena quartana.* Woolford B. Baker, Emory University.

A study of the metabolism of a colorless Euglena seemed desirable in order to secure further information regarding that of the forms possessing plastids bearing chlorophyll. Euglena quartana was selected for such a study. Observations were begun on organisms which had just emerged from cysts. At this time the body contains a large number of ribbon- or rod-shaped structures, peripherally located and uniformly distributed. In material fixed with Schaudinn's fluid, without acetic, the structures stain intensely with iron haematoxylin.

As the cycle advances the rods near the anterior end of the organism begin to show swollen areas at their tips or in their middle region. These areas increase in size and number until the rods themselves remain as thin threads or small granules. The swollen areas become paramylon bodies, which soon fill the entire body of the organism. Apparently, the paramylon is being elaborated by the deeply staining rods. As the body fills with paramylon the organism changes shape, becoming much enlarged anteriorly and more pointed posteriorly. Division of the body usually occurs before much paramylon is formed. When most of the rods have given rise to paramylon the organism encysts. Both nuclear and cytoplasmic changes during encystment are now being investigated.

*88. Relation of the volume of culture medium to growth rate of Euglena.* T. L. Jahn, University College, New York University. (Introduced by R. P. Hall.)

Large numbers of a pure line of Euglena sp. were obtained by continued growth in a buffered autotrophic medium in test tubes. By using a constant volume of culture medium and by varying the number of organisms (55 to 10,000 per cubic centimeter), the rates of growth for the various concentrations of Euglena were determined. Light and temperature were constant. The number of bacteria as determined at the beginning and end of each experiment was low, probably due to the absence of soluble organic compounds and to the effect of light. The flagellates were counted every twenty-four to thirty-six hours by means of a Sedgwick-Rafter counting chamber and a Whipple micrometer. Before each experiment the organisms were washed free of any autocatalyst that might have been present by repeated centrifuging with new sterile culture medium. At present no evidence of Robertson's positive allelocatalysis has been found. A negative allelocatalytic effect is indicated. The data on the applicability of the autocatalytic curve to euglenoid growth are not yet complete.

*89. The relation of density of population to rate of reproduction in Paramecium caudatum.* Walburga A. Petersen, Memorial Hospital, New York. (Introduced by W. C. Allee.)

When paramecia, washed or unwashed, were introduced singly and in twos into small volumes of bacterized medium (two or four drops), there was no acceleration in the division rate of the two-animal cultures; but when they were isolated singly, in twos, and in fours in twenty drops of bacterized medium the highest rate of division occurred in the cultures originally containing four animals, and the lowest rate occurred in the one-animal cultures. When forty-drop cultures were used, however, after twenty-four hours the four-animal cultures showed the most rapid division and the one- and two-animal cultures were about equal; but after forty-eight hours there was noticeable acceleration in both the two- and four-animal cultures. Animals isolated into twenty drops of bacterized medium infested with another ciliate showed approximately equal division in cultures originally containing one, two, and four paramecia.

When unwashed animals were isolated singly into varying volumes of sterile medium, the greater rate of division occurred in the smaller volumes in the first twenty-four hours. Animals, washed or unwashed, introduced singly into small volumes of conditioned medium, showed no greater division rate than control animals in unconditioned medium; but when isolated to twenty drops, there was noticeable acceleration in the conditioned medium.

An acceleration is present for limited animal-medium relations, which resembles in many aspects that described by Robertson in his work on allelocatalysis.

*90. The viability of Paramoecia and Euglenae in the digestive tract of cockroaches.* Robert Hegner, Johns Hopkins University.

These experiments have a bearing on two important problems: 1) the origin of the enteroparasitic habit of certain protozoa and, 2) the possible transmission of human protozoa by cockroaches. Rich cultures of Paramoecia and Euglenae were fed to cockroaches, and these insects were then examined at various intervals. It was found that Euglenae may remain alive within the crop of the cockroach for as long as five hours, but are unable to pass through the intestine and reach the rectum in a viable condition. Paramoecia were recovered from the crop of cockroaches at intervals of from thirty minutes to six and one-half hours, and in one case at the end of twenty-four hours, but no living specimens were found in the stomach. These experiments indicate that the trophozoites of Paramoecium and Euglena probably do not succeed in reaching the rectum of the cockroach in which parasitic flagellates and ciliates are normally to be found. It seems probable, therefore, that the trophozoites of other free-living protozoa suffer a like fate and that the colonization of the rectum of these insects must have taken place by the ingestion of cysts capable of resisting conditions in the crop, stomach, and intestine.

## COMPARATIVE ANATOMY

### *Read*

*91. Morphological studies of genetically different color patterns in Platypoecilus.* Myron Gordon, Cornell University. (Introduced by the Secretary of Section F.)

By a long series of breeding experiments, a genetical analysis of the complex color patterns in Platypoecilus maculatus has been made. Many in the dominant series of factors for various patterns are epistatic in effect. In time they have segregated out and have given rise to stocks, each of which is characterized by a single isolated pattern. By recrossing of these individual patterns, any combination may be obtained, success conditioned only by the genetic relationship of the various genes in terms of strength of linkage or multiple allelomorphism.

Morphological studies of isolated and compound patterns have been made of the developmental stages and of their final structures in adults. The effect of the interaction of genetic factors resulting in modifications of patterns has also been studied. Finally, the study of the effect of hybridization, i.e., Platypoecilus × Xiphophorus, upon specific characters of each has been made and certain changes detected; although, in general, genes of each species retain their own identity in the hybrid condition.

Genetic methods have proved valuable in separating apparently simple, superficially identical patterns. By a closer morphological study of isolated patterns it has been made possible to detect real differences otherwise obscured. Further interest is attached to this study due to the fact that all of the isolated patterns have been previously reported as occurring in nature.

*92. Observations on the fat-bodies in Amblystoma maculatum.* Gladys K. McCosh, Cornell University. (Introduced by the Secretary of Section F.)

In a very complete series of stages ranging from cleavage to those beyond the completion of metamorphosis, it has been possible to trace the development of the fat-body step by step. Its initial appearance is made relatively late in development at a time when the gonad is a clearly defined organ. Its origin "as a small bud of cells growing out medially from the hilus of the gonad" is confirmed. This bud consists only of mesothelial cells enclosed in a peritoneal sheath identical with that surrounding the gonad itself.

Shortly afterward the structural appearance of the gonad and fat-body is practically identical, since each contains numerous germ cells, some of which may be dividing. That the presence of germ cells in the fat-body is not due to a migration of these cells from the gonad, but rather to their development from the epithelium surrounding the fat-body, is supported. The potentiality of this epithelium to form germ cells is believed to be a temporary one, which is apparently lost after the deposition of lipoid material in the fat-body.

All the progressive changes in this organ are followed from a time when it possesses all the characteristics of an indifferent gonad until it loses any gonadal resemblances and becomes an organ distended with lipoid substances and consequently so increased in size that it extends far beyond the caudal limits of the reproductive gland.

93. *Types of cysts, calcific concretions, and pigmentation on the full-term foetal placenta of the pig.* CHARLES E. ABROMAVICH, JR., Johns Hopkins University. (Introduced by R. P. Cowles.) (Lantern; 7 min.)

A large number of full-term foetal membranes have been studied with the result that new information has been obtained concerning types of cysts, pigmentation, and calcific concretions. Heuser ('26) described cysts and pits arising as invaginations of the intervillous areas of the chorion. The walls of these cysts and pits are highly vascular and the contents in some instances are pigmented, in others pigmentation is lacking.

On the ends of the chorion there occurs another type of cyst, heretofore undescribed. This type likewise arises as an invagination of the intervillous areas of the chorionic surface. However, before invagination, at the site of the definitive cyst there is present an amorphous mass of detritus of questionable origin. This amorphous mass becomes embedded in the mesoderm between the chorion and allantois. The cysts gradually sink through the chorionic mesoderm and finally become free in the allantoic fluid. Indeed, shortly before term, dark brown bodies may be found in the allantoic cavity. Examination of the content shows it to be made up of cholesterol crystals, pigment granules, and degenerating cells. The pigment from general reactions has been identified as melanin rather than a lipochrome.

Deposits of calcium salts (especially the carbonate) have been observed in the full-term foetal placenta of the pig which were laid down along the courses of the blood vessels between the chorionic and allantoic membranes. This is by no means a frequent occurrence, since only a very few from the large number of specimens examined contained calcific concretions.

94. *Placentation of the Indian antelope (Antilope cervicapra).* CHARLES E. ABROMAVICH, JR., Johns Hopkins University. (Introduced by R. P. Cowles.) (Lantern; 8 min.)

Through the courtesy of Doctor Fox, of the Zoölogical Society of Philadelphia, opportunity has been afforded for the study of the foetal membranes and uterus of a ruminant heretofore undescribed. The uterus in question is of the bicornuate type possessing a rather long body. The right horn is very much larger than the left and contains the foetus, measuring 124 mm. in length. The left ovary contains a single corpus luteum. The foetal placenta or allanto-chorion extends through both uterine horns.

The chorionic surface is interrupted by patches of villi or foetal cotyledons. These foetal cotyledons fit into corresponding structures on the maternal placenta, the maternal cotyledons. There are two kinds of maternal cotyledons, those attached to the uterine surface by broad peduncles and those without peduncles. The cotyledons that are attached to the uterine surface by peduncles are dome-shaped and disc-shaped, sometimes somewhat concave. As a general rule, there is a paucity of cotyledons among the members of the deer family. In this specimen there is present a large number of cotyledons, reminding one of the placentation of the sheep and cow. The intercotyledonary areas, both uterine and chorionic, possess numerous small circular depressions. The depressions on the maternal side have uterine glands opening into them, while the depressions on the foetal side have a heightened epithelium. In the region of the umbilical cord the allantoic membrane is covered by numerous conical cysts of various sizes.

### *By demonstration*

### *95. A new type of nephridia in nemerteans.* W. R. Coe, Yale University.

In the paleonemertean genus Cephalothrix, as well as in Prostoma and Geonemertes among the Hoplonemertea, the excretory system consists of very numerous small groups of end bulbs, each with a separate efferent duct.

In a new species of Cephalothrix (C. major) from California the worms reach a size many times larger than in any other known species of the genus and possess an excretory system of remarkable complexity. More than 300 pairs of separate nephridia are found in an individual exceeding a meter in length. Each of these consists of three parts: *a*) the terminal organ, or end bulb, which is a multinucleate, mushroom-shaped structure bearing a wreath of cilia swinging in a thin-walled capsule situated close beneath the endothelium of the lateral blood lacuna; *b*) a narrow neck which leads to a broad convoluted tubule, from which, *c*) the slender efferent duct leads to the exterior of the body. The cytoplasm of the cells of the convoluted tubule is coarsely granular, with numerous inclusions, and is apparently comparable in structure with that of the main longitudinal canal in most other nemerteans.

### *96. Abnormal reproductive organs in Rana pipiens.* Bernice L. Maclean, Mount Holyoke College. (Introduced by Ann H. Morgan.)

This frog, which was killed on January 18, 1928, was an adult measuring 78 mm. from snout to rump. It appeared to be a male because of its enlarged thumb pads, but examination of the internal organs has disclosed variations from the male structure. The right testis is in no way atypical, but the left one is irregularly S-shaped. The wolffian ducts enlarge into seminal vesicles and enter the cloaca normally. The most unusual condition is found in the müllerian ducts. In Rana pipiens and some other species of frogs these persist in the male as rudiments, resembling the oviduct of the female anatomically and histologically, but ordinarily not exceeding 1.5 mm. in their widest part. In the individual under discussion, the ducts have the width, length, many convolutions, and uterine dilatation of the female. Histological examination of the testes has shown only normal spermatic tissue and neither healthy nor degenerating ova.

The ducts are like those of the female, with a ciliated epithelial lining throughout, and abundant mucous glands in the convoluted portion. The association of these essentially female structures with apparently active testicular tissue and the absence of ovarian tissue are perhaps explainable on the basis of late development of the endocrine element of the testis, or sex reversal.

*97. The leucocytopoietic organs of an Oligochaete, Pheretima indica (Horst).* James E. Kindred, University of Virginia.

The leucocytopoietic organs of Pheretima indica (Horst) are segmentally arranged. They are paired diverticula which project from the anterior surface of each septum from the twenty-fourth segment to the caudal end of the worm (seventy to seventy-five segments). They lie on each side of the dorsal blood vessel, and each organ is joined to its fellow of the opposite side by a fold of the septum. Histologically, the organs are composed of muscle cells, peritoneal epithelial cells, and leucocytes. The wall of each organ is covered externally and lined internally by a squamous epithelium. Between the epithelial layers there is a layer of muscular tissue continuous with the muscular tissue of the adjacent septum and the dorsal vessel. The lumen of the organ is continuous at its junction with the septum with the lumen of the perivisceral cavity. Internally, the lumen is divided into a series of canals which radiate from the centrally placed lumen. These canals contain leucocytes of several types, including lymphocytes, eosinophilic granulocytes, basophilic granulocytes, metachromatic granulocytes, and lamprocytes. Occasional chloragocytes may occur. The organs are termed leucocytopoietic, because there is evidence that the eosinophilic granulocytes originate here by a process of ripening of basophilic granulocytes, which in turn have originated from either lymphocytes (hemocytoblasts) or peritoneal epithelial cells.

*98. Ventral union in a bifid embryo of the earthworm, Sparganophilus eiseni.* Florence Hague, Sweet Briar College.

Serial sections demonstrate the relationships of setae, of digestive, nervous, and nephridial systems in this bifid embryo.

*99. Longitudinal sections of deer antlers, showing structure and growth during one season (Odocorleus virginianus).* C. V. Noback, Heads and Horns Museum, Bronx Zoölogical Gardens. (Introduced by G. K. Noble.)

Demonstration given at time of luncheon and inspection of the gardens, Thursday, December 27th.

*By title*

*100. The structure of the ductless glands in Alligator mississippiensis.* Albert M. Reese, West Virginia University.

A description is given of the gross and microscopic structure of the following glands of the American alligator: thyroid, thymus, hypophysis, adrenal, spleen, and pancreas.

A comparison is made between these glands after the prolonged fast of hibernation and after a period of active feeding.

*101. A new approach to the study of the ultimobranchial body.* Amy Grace Mekeel, Cornell University. (Introduced by the Secretary of Section F.)

In Amblystoma maculatum, as in Cryptobranchus, in which form there are also four pairs of external gills, a sixth pair of gill slits is laid down. These are temporary structures, are located caudad of the fourth branchial cartilages, and are distinct from the ultimobranchial body.

The lung anlagen antedate both the sixth gill slits and the ultimobranchial body. They outpocket from near the middle section of longitudinal grooves extending from the pharyngeal floor along the caudolateral wall of the foregut to the liver diverticulum. The anterior portion of each infolded lung groove is pinched off from the foregut, but presents a temporarily open end to the pharynx and is in alignment with a longitudinal groove in the pharyngeal floor.

The ultimobranchial body appears at the cephalolateral corner of this lung groove. It seems probable, when we recall the puzzling nature of this body, its persistent appearance in pulmonate vertebrates, its location near the level of the glottis, its relationship to the pericardial cavity, its blood supply from the pulmonary artery, and its general absence in teleosts, that this structure is closely associated with the lung and that its apparent relation to the caudal gill slit is due to spatial necessity.

If we consider the lung groove as representing the x gill slit, the ultimobranchial body may be a glandular derivative—perhaps a thymus body, or it may represent a vestige of the x-1 gill slit or possibly a combination of the two.

*102. The pigment granules of human head hair: a comparative racial study.* Leon Augustus Hausman, New Jersey College for Women (Rutgers University).

In human head hairs the structural characters of the cuticular scales and medulla are related to the diameter of the hair shaft, and not to race, as has been shown in an earlier study, but the characters of the pigment granules (size, color, placentation in the cortex, and patterns into which they are grouped) are correlated with the color of the hair shaft, and not with race or individual. Determinations of hair color (both grossly and under the microscope) were made with Ridgway's ''Color Standards and Color Nomenclature.'' It was further found that hair shafts of the same racial (and often of the same individual) origin vary greatly in pigmentation characters, as well as in characters of scale and medulla.

*103. An investigation of the age correlations of two structural elements of human head hair.* Elizabeth M. Wynkoop, New Jersey College for Women (Rutgers University). (Introduced by Leon Augustus Hausman.)

A study of two of the structural elements of the hair shafts of human head hairs from individuals ranging from one to seventy years shows that the size and form of the cuticular scales and the morphological type of the medulla are definitely correlated with the diameter of the hair shaft, and not with the age of the individual. The diameter of the hair shaft shows a rough correlation (subject to wide variation within limits) with the age group of the individual; its minimum diameter occurring in individuals between one and ten years and again between fifty and seventy years, and its maximum diameter in individuals between ten and fifty years.

104. *The growth of the kidneys in the cat embryo.* HOMER B. LATIMER, University of Kansas.

Both kidneys were removed from 138 cat embryos of unknown age, but ranging in body weight from 8 to 197 grams. A relatively small number of newborns is included in the above number and some smaller embryos are yet to be studied. The weights of both kidneys were plotted on body weight and on body length (nose-anus) and both curves are concave superiorly, although the second curve rises more rapidly toward the end. There is no sex difference in the weights of the kidneys. The range is from 0.039 to 2.48 grams.

The kidneys increase in weight more rapidly than the entire body, since they form on the average a little less than 0.6 per cent in the smallest embryos and over 1.0 per cent in the largest embryos. The percentage curve does not rise uniformly when either body weights or body lengths are used as abscissae.

The right and the left kidneys vary a good deal in weight, but on the whole they average about the same. The right kidney is higher, or more cephalad, in about 85 per cent; the left, in 6 per cent; and at the same level, in 9 per cent of the embryos. There seems to be no correlation between weight and position; the more cephalic kidney may be either the heavier or the lighter.

105. *The development of the vertebral column of Anura and its bearing on the law of cephalocaudal differential growth.* W. C. SENNING, Cornell University. (Introduced by the Secretary of Section F.)

A study of the development of the vertebral column gives no evidence in support of the view that the first vertebra of Amphibia represents the fused epistropheus and atlas of other forms. A study of the urostyle does not support the view that this structure represents fused caudal vertebrae. The vertebrae forming the urostyle belong to the trunk region. Such views evidently arise from an effort to cross-homologize the vertebrae of one form with those of another.

The development of the component parts of a vertebra and the differentiation of these parts into the definite vertebral column follow the cephalocaudal sequence as exhibited by many other structures in the vertebrate body. Especially is this true of certain of the processes of the vertebrae. There seems to be abundant structural evidence that the difference in degree of development of the anterior end over the caudal end is merely a time difference. Apparently ossification of the vertebrae, which, at least in the human, does not strictly follow a cephalocaudal sequence, does so in the Amphibia studied.

106. *Sexual dimorphism of the symphysis pubis of the bat Myotis lucifugus (Miller).* C. HAMRE, R. K. MEYER, AND S. J. MARTIN, University of Wisconsin. (Introduced by Frederick L. Hisaw.)

The pubic bones of the sexually mature female bat Myotis lucifugus (Miller) are united by fibrous ligaments. A study of the symphyses of the foetal and sexually immature female bats demonstrates that the symphysial unions are of hyaline cartilage surrounded by fibrous tissue. These seemingly contradictory data are interpreted to indicate that the pubic symphysis of the sexually immature female changes to the condition described for the mature female during the attainment of sexual maturity. During pregnancy the pubic ligaments increase greatly in thickness and in width, indicating that these changes are of importance in parturition. The metamorphosing of the immature female symphysis and the enlargement of the pubic ligaments of the pregnant female suggest that the symphysis is under the control of the ovarian hormones as demonstrated in the guinea-pig. (Hisaw, in press.)

The symphyses of immature and mature male bats are composed of hyaline cartilage surrounded by fibrous connective tissue, as in the immature female. In the case of the older males, the cartilage may be replaced by bone at the anterior end of the symphyses and the pubic bones united by interlocking bone trabeculae. The same type of sexual dimorphism of the symphysis pubis as found in the bat Myotis lucifugus has been described for the guinea-pig by Hisaw.

*107. Studies in the anatomy and natural history of the devil-fish, Manta birostris.* L. Hussakof, American Museum of Natural History.

This paper is based on materials in the American Museum of Natural History, consisting of an entire small Manta, a cast of a full-grown one, and preserved portions of several large ones; also field notes made by the writer on the west coast of Florida.

The paper will deal with three aspects: 1) External morphology, and comparison with the related Mobula (including the first accurate figure of Manta); taxonomy, distribution. 2) Natural history, with the special object of clearing up conflicting accounts about certain habits. 3) Anatomy: The structure and mode of operation of the cephalophores; their morphologic relation to the fins, and comparison with the same organs in Mobula. In the dentition it was found from five dental plates of different-sized specimens that the number of rows of teeth is not constant for the species, but varies with age—from 212 rows in a specimen 7 ft. wide to 371 rows in one 17 ft. wide. Description of the teeth and shagreen. Morphology of parts of the skeleton and of some of the internal organs, especially structures distinctive of the mantid rays.

## MISCELLANEOUS

### *Read*

*108. The indexed continuation card catalogue of the bibliography of fishes.* E. W. Gudger, American Museum of Natural History. (10 min.)

I was editor of the third and index volume of Dean's Bibliography of Fishes, embracing the literature through 1914, which was published in November, 1923. At the meeting of the American Society of Zoölogists, January 1, 1925, I explained my purpose of keeping the Bibliography going in the form of an indexed card catalogue and asked that fish papers be sent in to me for immediate cataloguing. With the generous support of Dr. Bashford Dean and the prompt response of many zoölogists, this plan has gone steadily forward. An immense amount of work has been done, especially in checking lists of titles from unusual sources, and we now have approximately 8000 titles—at least 50 per cent more than are to be found in the Zoölogical Record from 1915 to date. This catalogue is a widely comprehensive one, planned to embrace all subjects, however obscure, dealing directly or indirectly with fishes. Obviously, we cannot send out this data by mail, but every facility is afforded visiting scientists to use the cards. During the past year there have been more such visitors than ever before. Interested zoölogists are invited to examine the catalogue while in New York and are asked to send in their papers dealing with fishes as soon as published, that these may be catalogued in advance of the checking of the Zoölogical Record and other annual bibliographies which appear some years later.

*109. The living organism and the new physics.* WM. G. H. BREHMER. (Introduced by the Secretary of Section F.)

One of the most far-reaching achievements of modern science has been the experimental demonstration of the intensely dynamic and complex nature of the ultimate particle entities of our 'material' environment. This experimentation gives rise to the idea that the atoms are themselves of the nature of elemental organisms. Such a concept leads to the interesting question whether the living organism might not be of the nature of a tremendously advanced 'social community' of energized particles, each one striving to advance itself in the process of particle socialization, even as we strive to advance ourselves in the process of human socialization.

In this paper an attempt is made to outline various stages in an orderly process of particle organization such as might be expected to occur in so remarkable a 'particle society' as water. It is shown that in certain rare particle organizations the smallest, most energetic, most 'social' particles would tend to concentrate themselves, with gathering momentum, into a central, dominant group (eventually the cell nucleus). This dominant group would become the center of organic development. It would tend in due time to thrust itself toward new and favorable sources of environal energy. In the most progressive cases it would tend to establish itself in a 'head end,' and organize the entire particle society into efficient systems to further the process of particle socialization. In its higher interpolations this 'social' theory gives clear insight into the nature of that peculiar genius of the living organism to seek and select its developmental experiences.

*By demonstration*

*110. A new staining dish for the handling of cover-slip preparations.* ROBERT H. BOWEN, Columbia University.

*111. A paraffin oven for individual student use in courses involving histological technic.* ROBERT H. BOWEN, Columbia University.

*112. Device for the control of temperature in localized areas of eggs and embryos.* E. VAN SLYKE, University of Pittsburgh. (Introduced by Robert T. Hance.)

This device is designed for the heating of microscopic areas of embryos. It is used in connection with a microdissection apparatus and is arranged so that the temperature may be very accurately controlled.

*113. A method of obtaining blood directly from the branchial circulation of Necturus.* HARRY A. CHARIPPER, New York University. (Introduced by Alden B. Dawson.)

*By title*

*114. Blue or black stains with haematoxylin.* ELBERT C. COLE, Williams College.

Careful study has shown that such preservatives as sodium hydrosulphite or potassium pyrosulphite are harmful when used in stock solutions of haematoxylin containing more than a trace of water. Accordingly, such solutions have been replaced by the usual solutions of haematoxylin crystals in absolute alcohol in the technique already described by Cole (Science, vol. 64, pp. 452–453). Even freshly prepared stock solutions yield excellent results in the following technique, without a tedious process of ripening.

Ten drops of stock 2 per cent haematoxylin solution are added to 5 cc. of tap-water to which has been added a small crystal of disodium hydrogen phosphate. A dropping bottle is used. The solution turns a bright pink, which deepens to a red within a few minutes. The stain is ready for instant use and retains its staining power for about twenty-four hours. A trace of ammonium hydroxide hastens the ripening process. Tissue sections mounted on slides and mordanted with 5 per cent iron alum are flooded with the ready stain. Staining is rapid and intense. Differentiation is accomplished in very dilute iron alum, in 0.1 per cent HCl, or in 0.1 per cent $HNO_3$. This gives a sharp nuclear differentiation. Tissues are then 'blued' in water containing a trace of ammonium hydroxide. The resulting picture is in brilliant blue. Posttreatment in a bath of 0.01 per cent chromic acid changes the stain to a rich black. Tissues are then washed, dehydrated, cleared, and mounted in the usual way.

## CYTOLOGY

*Read*

*115. Normal variations in red blood-cell counts and haemoglobin determinations.* CHRISTIANNA SMITH, Mount Holyoke College. (Introduced by Ann H. Morgan.) (Lantern; 15 min.)

Total red counts and haemoglobin determinations were made every half-hour for four- or eight-hour periods on two subjects in intermenstrual periods. One hundred and seventy-three counts were made on subject A and ninety-six on subject B. For subject A the mean was 4,115,680 ± 19,681; the standard deviation, 382,620 ± 13,873; the coefficient of variation, 9.3 ± .34 per cent. For subject B the mean was 4,299,688 ± 22,065; the standard deviation, 320,533 ± 15,603; the coefficient of variation, 7.4 ± .36 per cent. The number of haemoglobin determinations on subject A was 108 and on subject B, 56. The mean for subject A was 10.4 ± .014 grams; the standard deviation, .22 ± .01 grams; the coefficient of variation, 0.2 per cent. For subject B the mean was 10.4 ± .045 grams; the standard deviation, .5 ± .03 grams; the coefficient of variation, 0.47 per cent.

There was no afternoon rise and no change after meals. The fluctuations in the numbers of reds during the day were due probably to errors in technique. The sum of the probable error of the mean, the standard deviation and its probable error amounted to 832,348 in A and 816,542 in B. In A the difference between the high and low points in a day was usually 500,000 or 600,000 cells and in B, 700,000 or 800,000. As the haemoglobin determinations were so much more constant, the data indicate that isolated determinations of haemoglobin are of more value than isolated total counts and changes in haemoglobin content more significant.

*116. Connective-tissue structure of the ganglia of the earthworm.* M. THELMA HOLMES, Syracuse University. (Introduced by W. M. Smallwood.) (10 min.)

A restudy has been made of the cellular connective-tissue structure of the ganglia of Lumbricus terrestris, with special emphasis upon the structure of the giant-fiber sheath. There are two types of connective-tissue cells in the ganglion. One type is smaller, with a minute amount of cytoplasm; while the other has a less densely staining nucleus and a greater cytoplasmic content. Both types are readily distinguishable from nerve cells because of their size, nature of their processes, staining reactions, and position in the ganglion.

The sheath of the giant fibers is made up of the cell bodies of neuroglia cells and of the processes of such cells. These cells are found not only in the sheath as it extends longitudinally in the cord, but they are definitely the contributing elements of the septa of the dorsal giant fibers and hence play an important part in synapse. Experiments with the regeneration of the nervous system support this interpretation.

*117. The membranous structure of striated muscle.* H. N. BAKER, H. C. MCPHEE, AND PAUL E. HOWE, U. S. Department of Agriculture. (Introduced by the Secretary of Section F.) (Lantern.)

Evidence is presented to show that the fibrillae of striated muscle are not fixation precipitations, but are very delicate membranous tubes filled with semifluid protoplasm. The cross striations peculiar to muscle are apparently a feature of the fibrillae and are membranous with adhering granules. These fibrillae differ in consistency from the center of the fiber to the outside. This fact is demonstrated by the dissolving power of certain salts, by the aid of which the central part of the fiber appears to be completely extracted. This depletion of the inside of the fiber leaves a characteristic doughnut-shaped disc. The sarcolemma is an entity with a different composition than the connective tissue proper, as demonstrated by a different staining reaction with Mann's eosin-methyl blue. The adhering endomysium differs in its composition from the other connective tissue.

The phosphates are much more selective in their action than the other salts; walls of the fibrillae are left intact while sodium chloride clears out the interior of the fiber. These observations were made after guinea-pig, beef, and chicken muscle had been extracted with various volume molar concentrations of NaCl, mixtures of $KH_2PO_4$ and $K_2HPO_4$, $NH_4CL$, $NH_4OH$, KCNS, and KI, in which as high as 85 per cent of proteins had been extracted, on the basis of nitrogen determinations. The hydrion concentrations enabled differential staining after the buffer phosphates were used to extract.

*118. Further observations on the ovarian eggs of Fundulus.* MARY J. GUTHRIE, University of Missouri. (12 min.)

Ovaries from Fundulus heteroclitus and F. majalis were stained with vital dyes. During the early growth period of the oocytes the cell components appeared in the same sequence and occurred in the same positions that I have described from a study of preserved material. Nucleoli were amoeboid, vesiculated, and peripheral in arrangement in small oocytes, but none was observed to migrate from nucleus to cytosome. Mitochondria, which were stained by Janus green, and pseudoyolk granules, which were stained by neutral red, were at first localized near the nucleus and later became dispersed and decreased in number. Neutral fat-drops which stained red with sudan III, scharlach R, and Nile-blue sulphate were very small and occurred in clumps. The proteinaceous yolk vesicles were not homogeneous, but each consisted of a substratum in which one or many spheres of a different composition were dispersed. These dispersed spheres within the proteinaceous yolk vesicle stained a dull orange to brick-red color with neutral red and became blue when treated with Nile-blue sulphate, methylene blue, and brilliant cresyl blue. The substratum stained very faintly with the dyes used. Deeper vesicles colored more quickly and more intensely than did the peripheral vesicles of any particular oocyte. Within a certain size range, vesicles in larger cells stained sooner and more heavily than those in smaller cells. The proteinaceous yolk spheres did not lose their individuality when the cell membrane was ruptured. Oocytes during the later growth period became difficult to stain and observe.

### *119. Cytological studies on the spinning glands of Platyphylax designatus Walker (Trichoptera): respective rôles played by the nucleus and the Golgi apparatus during secretion.* H. W. BEAMS AND C. F. WU, University of Wisconsin. (Introduced by George Wagner.) (Lantern; 7 min.)

The results of a cytological study of the spinning glands of the larvae of Platyphylax designatus Walker are as follows:

1. A general increase in diameter is accompanied by activity in the spinning glands of the larvae of Platyphylax designatus Walker.
2. In the spinning glands of the larvae of Platyphylax designatus Walker the nucleoli migrate into the cell body, enlarge, undergo dissolution, setting up streaming effects in the cytoplasm and forming at least a part of the secretory products of the cell.
3. In the outer area of the gland appear numerous vacuoles which were found to be greater in size and number with increased period of activity. These we interpret to be the remains of dissoluted secretory inclusions.
4. There exists in the spinning glands of Platyphylax a true Golgi apparatus, which presents itself in the form of rings, loops, or comma-like structures.
5. The Golgi apparatus is evenly distributed throughout the gland and shows no apparent hypertrophy during the different periods of physiological activity of the gland.
6. The Golgi apparatus shows no apparent relationship to the secretory phenomenon, such as direct connection with the secretory inclusions, definite fixed polarity, or hypertrophy during activity of the gland.
7. Only a small amount of the Golgi material is extruded from the cell body with the products of secretion.

*120. Phenomena associated with the distribution of the plastidome in multinucleate cells of Ricinus.* ROBERT H. BOWEN, Columbia University. (Lantern; 10 min.)

Certain cells in the plerome of the root tips of Ricinus communis regularly become multinucleate by the failure of cell-plate formation at the conclusion of an otherwise normal mitosis. Such cells have at first two nuclei, which subsequently divide simultaneously to produce four nuclei, etc. In normal mitosis there is set up in all of the younger plerome cells a constant topographical relation between the usually elongate proplastids and the spindle poles. In the binucleate cells, approximately half of the proplastids become associated with each prophase nucleus, and each group then passes through the same phases of orientation and ordered distribution which are characteristic of the mononucleate condition. Although the cell contains two nuclei, each nucleus behaves as though it were independent of the other. The relation between the proplastids and a spindle pole is so similar to the association sometimes set up in animal cells between a centriole and chondriosomes as to suggest that the mechanisms of mitosis in plant and animal cells may not be so different as is usually thought. It also raises further doubts as to the validity of the explanation which ascribes polar orientations of cytoplasmic-formed elements to a merely fortuitous association with the cytoplasmic currents set up during mitosis.

*121. Regeneration in nemerteans.* W. R. COE, Yale University. (Lantern; 12 min.)

Two species of nemertean worms, Lineus vegetus of the Pacific coast and L. socialis of the Atlantic coast, exhibit amazing capacities for the regeneration of body fragments spontaneously produced or of minute pieces cut from the body. By successively cutting the regenerated minute worms, miniatures of less than one ten-thousandth of the original size have been obtained.

Any piece of the body, if of sufficient size, with the exception of fragments taken anterior to the brain and those containing a portion of the brain but lacking any part of either cerebral sense organ, is capable of regenerating. From a single worm 100 mm. long several hundred regenerating individuals of minute size can be simultaneously produced, if suitable transverse and longitudinal cuts are made. Various types of twins result from partial longitudinal sections.

Pieces containing no trace of the original digestive system restore the new midgut by the proliferation of mesenchyme cells from the body parenchyma, but these are not derived, as has been claimed for a closely related species, from the wall of the proboscis sheath. The foregut is restored by an ectodermal invagination similar to that which occurs in larval development, and not, as has been asserted, by a differentiation of the midgut.

The cerebral sense organs, brain, and proboscis epithelium, as well as the foregut, are restored by invaginations of the new ectoderm formed by the migration and proliferation of formative cells derived mainly from the basal layers of the original epidermis. Phagocytosis by parenchyma cells, followed by active migration, transfers the substance of the old tissues to the area of proliferation.

*By demonstration*

*122. The reaction of the 'segregation' apparatus of the leucocytes of the blood of Necturus to neutral red and to prolonged exposure to osmic acid.* ALDEN B. DAWSON, University College, New York University.

*123. Hemocytopoiesis in the African lung-fish.* H. E. JORDAN AND C. C. SPEIDEL, University of Virginia Medical School.

The blood of the African lung-fish is of special interest because of the enormous size of the cells and the wide variety of granulocytes. While these blood cells resemble those of urodele blood, the liver lacks the characteristic perihepatic shell of granulocytopoietic tissue of the salamander.

Granulocytes arise in spleen, intestine, and kidney from lymphoid hemoblasts, or from reticular cells. Four main varieties may be recognized: 1) eosinophils with coarse spherical granules; 2) eosinophils with fine granules (special granulocytes); 3) basophils with metachromatic (purplish) granules, and, 4) 'meta-eosinophils' with coarse spherical granules, which may be colored light red by Giemsa's stain or light lilac, green, yellow, or they may be practically colorless. These 'meta-eosinophils' are thought to be senile stages of the ordinary eosinophils with coarse granules. They appear to be homologous to the chromophobic granulocytes described by us in the turtle spleen and thymus. In the early stages of eosinophil development from hemoblasts, fine mixed basophilic (bluish) and eosinophilic granules may be present.

Erythrocytes and thrombocytes differentiate in the spleen and in the general circulation from lymphòid hemoblasts. Senile erythrocytes are numerous in the spleen and general circulation. To a moderate extent they are ingested in the spleen by monocytes.

The lung-fish affords excellent material for a comparison of the blood cells under normal conditions and under conditions of prolonged estivation.

*124. A peculiar type of granulocyte in the spleen and thymus of the turtle.* H. E. JORDAN AND C. C. SPEIDEL, University of Virginia Medical School.

The granulocytes in the spleen of the turtle include eosinophils with spherical granules, eosinophils with bacillary granules, basophils, and peculiar granulocytes with chromophobic granules. The chromophobic granules are generally spheroidal, and they often appear swollen or blob-like, as if fluid had been imbibed. With Giemsa's stain they range in color from light red or lilac to colorless. The nucleus is spherical and is usually located at one pole of the cell. The cytoplasm in many cases is somewhat skeletonized. Genetically, this cell is believed to represent a late stage of the typical eosinophil with spherical granules.

In the thymus, nests of granulocytes are present of the two eosinophil varieties and also of the peculiar type described above. Some of these cells contain basophilic granules as well as eosinophilic or chromophobic granules. All three kinds of granules may be found in a single cell. Such cells probably represent abortive eosinophils. The red granules are those which have ripened properly, the chromophobic granules are those which have become senile (either passing through, or failing to pass through, an eosinophilic stage), and the basophilic granules are those which have failed to ripen, thus retaining the more primitive basophilic condition.

Early stages in the development of eosinophils in the thymus from hemoblasts show cells which contain fine mixed basophilic (blue) and eosinophilic granules.

125. *Connective-tissue structure of the ganglia of the earthworm.* M. THELMA HOLMES, Syracuse University. (Introduced by W. M. Smallwood.)

126. *Hereditary rodless retina of the house mouse.* CLYDE E. KEELER, Bussey Institution.

127. *The so-called secretory canaliculi of the human thyroid gland.* LESLIE B. AREY, Northwestern University Medical School.

*By title*

128. *The chromosome cycle in the rotifer, Asplanchna amphora.* DAVID D. WHITNEY, University of Nebraska.

The diploid number of twenty-six chromosomes was found in the mature parthenogenetic female-producing eggs and also in the somatic cells of the female embryos developing from such eggs. In the maturation stages of a few of these eggs the chromosomes were markedly larger than in the corresponding stages of the majority of the eggs. Whether this size difference of the chromosomes is correlated with male- and female-producing individuals has not been determined. The mature parthenogenetic male-producing eggs contain the haploid number of thirteen chromosomes and this number was found also in the somatic cells of the young male embryos. The mature sexual eggs contain thirteen chromosomes. In spermatogenesis the secondary spermatocyte divisions are usually omitted, and the secondary spermatocytes develop directly into the motile spermatozoa containing thirteen chromosomes. A few, however, of the secondary spermatocytes divide, forming spermatids containing fewer than thirteen chromosomes. These cells develop into the non-motile and rudimentary spermatozoa. The motile spermatozoa containing thirteen chromosomes unite with the parthenogenetic male-producing eggs containing thirteen chromosomes, thus producing the fertilized eggs with the diploid number of twenty-six chromosomes. These fertilized eggs develop into female-producing females which reproduce parthenogenetically.

129. *The rôle of the mitochondria in the formation of milk in the mammary glands of the albino rat.* H. W. BEAMS, University of Wisconsin. (Introduced by George Wagner.)

These observations agree with those of Hoven's (Anat. Anz., Bd. 39) for the most part as to the position, number, and appearance of the mitochondria in the mammary gland. The mitochondria are usually filamentous or rod-like structures, generally oriented in the direction of the main axis of the cell. There is a noticeable increase in the mitochondria of the rapidly secreting gland over that found in the non-hypertrophied gland. Definite small granules, which take up the same stain as the mitochondria (Regaud method), may be observed in the lumen end of the secreting epithelial cells and also in the lumen of the actively secreting gland. A number of the secretory granules apparently may fuse in the lumen of the gland to form large droplets of secretion. Careful scrutiny indicates a possible topographical relationship between the chondriosome and the small secretory granules. This condition suggests that some of these small granules may be directly formed from the chondriosomes by a process of synthesis, while others give the appearance of a direct breaking-up of the filamentous structures into granules which may be extruded from the cell with the products of secretion. Direct evidence to demonstrate Hoven's contentions that some of the granules go to form fat and some to form casein of the milk is lacking. These observations point to the assumption that in the mammary gland the mitochondria do play a part in the mechanics of secretion.

*130. The reaction of the 'segregation' apparatus of the leucocytes of the blood of Necturus to neutral red and to prolonged exposure to osmic acid.* ALDEN B. DAWSON, University College, New York University.

In a previous paper (Dawson, '28) the possible identification, in the erythrocyte of Necturus, of the 'segregation' apparatus with the Golgi apparatus was suggested. The same methods, supravital staining, prolonged exposure to osmic acid in a hanging drop, and the technic of Nassonov-Kolatchev have been applied in a study of the leucocytes of Necturus. In thrombocytes, lymphocytes, monocytes, and neutrophilic leucocytes the globules which stain readily with neutral red are also blackened with osmic acid. Less convincing results have been obtained with the eosinophilic and basophilic leucocytes, chiefly due to the large size and the staining reactions of the specific granules of these cells. In resting lymphocytes, monocytes, and neutrophilic and eosinophilic leucocytes the arrangement of the granules or globules is profoundly influenced by the presence of the centrosphere. They are either clustered about this area or arranged radially from it as a center. During ameboid activity the formed constituents of the cytoplasm are not arranged according to any definite pattern. In the thrombocytes the granules of the 'segregation' apparatus are usually bipolar and resemble closely those of the erythrocytes.

*131. Yolk- and fat-formation in the egg of Patella vulgata, L.* HOPE HIBBARD, Oberlin College.

The mature egg is peculiar in being crammed full of fat-spherules of uniform size and resembling yolk in distribution, among which are numerous smaller true yolk platelets. This proteic yolk is easily overlooked, and may be entirely absent after certain fixatives. It is evident in fresh material and after Dietrich-Parat or Flemming fixation. It is vitally stainable from its earliest appearance in the periphery of the cytoplasm until the egg is nearly ripe. At maturity most of the yolk is unstainable. In the veliger larva the rehydration of the yolk in digestion renders it again stainable.

The fat-globules arise in minute groups of droplets in the cytoplasm of small oocytes, increase in number and size until they occupy the greater part of the volume of the egg. They may be stained pink with Nile-blue sulphate in fresh material, orange-red in sudan III, black in osmic acid, are dissolved out by ordinary embedding methods, and are not at any time during their history stainable vitally by neutral red or Janus green.

Impregnations with osmic acid or silver nitrate give either rings and crescents (dictyosomes) made from incompletely washed-out fat-globules, or a more or less complete blackening of the true yolk. Either of these formations could be misinterpreted as the 'Golgi apparatus.' Elaborate nucleolar phenomena accompany the earlier stages. This description is at variance with that of Ludford (J. R. M. S., '21); resembles other cases of vitellogenesis described by myself; further confirms Parat's Golgi-apparatus theory.

### 132. *The formation of 'pigeon milk.'* H. W. BEAMS AND R. K. MEYER, University of Wisconsin. (Introduced by Frederick L. Hisaw.)

The formation of so-called 'pigeon milk' in the crop of male and female pigeons to nourish their young has been frequently described in text-books as a secretory process. It has been demonstrated that this process is not a secretory one, but involves an enormous hypertrophy of the stratified squamous epithelium which is localized in two lobes occupying a lateral and posterior position in the crop. Soon after the start of incubation the hypertrophy of the epithelium begins by rapid mitosis of the deeper cells. The cells nearest the basement membrane and especially around the capillaries, by continued division, become eventually cut off from their nutrition. This process throws the epithelium into large folds. This hypertrophy may be compared in some respects with that in the sebaceous glands with the exception that here the cells do not break down, but are desquamated in masses into the lumen of the crop and transferred in toto to the crop of the young squab. Indirect evidence indicates that the causation of this phenomenon is supplied by glands of internal secretion.

Histological examination of the material in the crop of the squab presents a similar picture as that in the lobes of the parents, with the exception that the cells show degenerative changes. Cytological analysis of the cells of the hypertrophied crop shows that surrounding the nucleus there are small spherical vacuoles which bear an apparent relation to the rod-like chondriosomes, and the contents of which give a fat-reaction to osmic tetraoxide.

### 133. *Histological changes in the mucosa of the enteron of the snapping turtle, Chelydra serpentina, Linn., following prolonged inanition.* EILEEN M. MILLER, New Jersey College for Women (Rutgers University). (Introduced by Leon A. Hausman.)

Normal histology of enteron mucosa in Chelydra serpentina does not differ essentially from Alligator and Testudo, as described by Reese, Kahle, and Staley. Two turtles were deprived of food, not water, for six and eighteen months, respectively. Changes include: utilization of normal liver fat; increased number of 'pigment islands'; confluent and shrunken hepatic cords; congested capillaries; distended gall bladders; enormous 'syncytial areas' in liver and throughout connective tissue in mucosa and submucosa of every region studied (excepting the esophagus); hypertrophy of connective and lymphatic tissue; general gland-cell atrophy; some submucosal fatty degeneration; general shortening of width of epithelium, and its complete loss over certain areas with the appearance of the débris in the lumen; decrease in blood-cell count (though blood parasite is an additional factor); and persistence of goblet cells. 'Syncytial areas' develop from contiguous cells, with granular cytoplasm and faintly staining nuclei, to syncytial masses with curved strands of brilliant, yellow, more highly refractive substance in center of aggregate. Areas become vacuolated; nuclei, more pycnotic in character, are massed at the periphery. The yellow strands opening out forming spheres containing fluid and occasionally densely chromatic material. 'Syncytial areas' are generally found surrounded by connective and lymphatic tissue. These studies were made at the suggestion of Dr. William H. Cole, Clark University. A similar investigation is in progress with 'snappers' starved for longer periods of time, also with Chrysemys picta for a comparison.

*134. Regeneration of the giant fibers in the earthworm.* W. M. Smallwood, Syracuse University.

During the past year neurological methods have been applied to the regenerating nervous system of the earthworm, and this method of approach will be continued. There seems to be a clear order in which the parts regrow. The connective tissue of the nervous system is the first to differentiate, but is quickly followed by the giant fibers. The nerve cells which send their processes into the giant fibers take a differential stain in the Bielchowsky-Patten technique. The nerve fibers in the body wall are very clearly defined at the period when the muscles and hypodermis are becoming recognized as distinct tissues.

## EMBRYOLOGY

### *Read*

*135. The cilia of the vertebrate oviduct in relation to the transport of spermatozoa.* G. H. Parker, Harvard University. (10 min.)

The oviduct of the painted turtle measures about 15 cm. in length. The whole interior of the duct is ciliated. These cilia form two systems, one that covers most of the inner face of the duct and whose direction of stroke is toward the oviducal outlet—that is, away from the ovary (abovarian system), and a restricted system in the form of a narrow tract extending throughout the length of the duct with cilia beating toward the ovary (proovarian system). Living spermatozoa can make no headway up the oviduct against the beat of the abovarian cilia. Hence there is no ground for assuming that rheotaxis plays any part in the migration of the sperm. Neither does the muscular activity in the oviducal wall nor ciliary reversal contribute to this end. The spermatozoa are apparently transported from their region of deposit by the male near the oviducal outlet to the neighborhood of the ovary solely by the system of proovarian cilia, and this transportation of the sperm is doubtless the function of the proovarian cilia. The abovarian cilia keep the oviduct clear and help in part to move the eggs. In the pigeon these two systems of cilia are also present. The condition in mammals remains to be ascertained.

*136. A preliminary study of the function of the epididymis.* William C. Young, University of Chicago and Brown University. (Introduced by Carl R. Moore.) (10 min.)

Beyond the rôle of the epididymis in sperm storage and its secretory activity but little is known about the functional relationship between this organ and its contents, the spermatozoa, and about the changes undergone by spermatozoa during their residence in it. The suggestions have been made: 1) that sperm are nourished by the epididymal secretion; 2) that the secretion preserves sperm vitality during their residence in it; 3) that the secretion has buffer properties which protect sperm against the acidity of the female genital tract; 4) that sperm attain full maturity and are strengthened in consequence of some action of its secretion; 5) that substances circulating in the blood which are toxic to sperm are in some way destroyed; and, 6) that the organ is resorptive as well as secretory.

As the first part of a comprehensive study, experiments were performed to test the theory that sperm attain full maturity in consequence of some action of the epididymal secretion. No evidence was obtained in support of the theory. On the contrary, the results indicate that sperm become strengthened and attain full maturity independently of any specific action of the epididymal secretion. It is suggested, therefore, pending the investigation of other aspects of the subject, that the epididymis is a reservoir for sperm in which important processes of sperm development which start while the sperm are contained in the testis are free to continue because of the favorable environment present in the epididymis.

*137. The origin of the first cleavage amphiaster in Echinarachnius eggs.* Henry J. Fry, Washington Square College, New York University. (Lantern; 10 min.)

It is assumed that cleavage amphiasters arise in two radically different ways. The first cleavage figure is supposedly formed when the preexisting sperm aster draws apart into two portions; the origin is 'partitional'; there is structural continuity between the old and new configurations. On the other hand, each later cleavage figure arises about two foci that appear within the center of a preexisting aster (of the preceding amphiaster) which later disintegrates; the origin is 'endogenous'; there is no structural continuity.

In Echinarachnius eggs the late sperm aster is structurally differentiated into a 'male' side which has clear rays and lies between the edge of the egg and the male chromatin; and a 'female' side which lies opposite and has vague rays. The first cleavage amphiaster arises as a new structure within the sperm aster at two foci lying in a line that is at right angles to that passing through the 'male' and 'female' sides. The outer ends of the old rays of the disintegrating sperm aster persist in the peripheral part of the egg long after the new-formed rays of the amphiaster have appeared.

The first cleavage aster of Echinarachnius, therefore, arises 'endogenously' in the same way as do later cleavage asters, and not in the exceptional manner by 'partition' as usually described. Should this result be found valid for other forms generally, it would clarify the situation morphologically for an eventual physicochemical explanation of the origin of cleavage amphiasters.

*138. Early embryonic differentiation of the chick hypophysis in chorio-allantoic grafts.* Kathryn Stein, University of Chicago. (Introduced by B. H. Willier.) (7 min.)

The hypophysis, or the region destined to give rise to it, together with varying amounts of adjacent tissue, including brain tissue, was transplanted from chick embryos to the chorio-allantoic membrane of hosts of eight or nine days' incubation. Of twenty-five grafts of implants in which the hypophysis was morphologically indicated at the time of grafting (twenty-two somites to five days) twenty-one showed hypophyseal tissue. Of nineteen from younger donors (twenty hours to twenty-one somites) only seven contained such tissue. The differentiation appeared similar to that in the normal embryo of approximately the same age. The small percentage of positive cases from young donors may perhaps be explained on the assumption that the organization of the hypophysis during this period was not definitely fixed, and consequently that hypophyseal tissue appeared only where relationships most nearly normal were maintained.

Attempts were made to separate the hypophyseal (or presumptive hypophyseal) ectoderm from brain tissue and to implant oral-plate region and forebrain region from the same donor on separate hosts. Of thirty grafts, only four of the latter type and none of the former showed hypophyseal tissue. This suggests that the presumptive hypophyseal cells are localized in the ectoderm closely applied to forebrain just anterior to oral-plate ectoderm. A process similar to the normal infundibular process was observed in most of the grafts containing hypophyseal tissue. Further study of brain structure in grafts lacking hypophysis and further experiments are necessary before statements as to correlative differentiation can be made.

### *139. An experimental study of the origin of the adrenal gland in the chick embryo.* B. H. Willier, University of Chicago. (Lantern; 8 min.)

By means of chorio-allantoic grafts of the isolated wolffian body, it is possible to determine the time of origin and to some extent the source of the two primordia of the adrenal gland.

Isolations were made at, 1) four days, approximately the time when cells from the primary sympathetic ganglia have first made contact with the cortical primordium, a derivative of the coelomic epithelium, and, 2) eight or nine days, when the so-called primordium of the medullary cords is represented by a large sympathetic ganglionic mass which, according to descriptive embryology, is derived from both the primary and secondary ganglia. In either case an adrenal is formed in the graft. It is a definitely circumscribed organ with well-differentiated medullary and cortical cords. The adrenal formed from a four-day implant is fully as well differentiated as one from a nine-day implant.

These experiments show that the essential components for adrenal formation are already localized early in the fourth day. The sympathetic cells just medial to, and perhaps mingling more or less with, the cortical rudiments at four days apparently really constitute the primordium of the medullary cords. Furthermore, cells from the secondary sympathetic ganglia are not essential to the formation of medullary cords, since the secondary sympathetic system arises somewhat later than the time of isolation of the implant on the fourth day. In the light of these results the relation of the large sympathetic ganglion contiguous with the cortical primordium on the ninth day to medullary-cord formation is obscure.

### *140. Development of the thyreoid gland in Necturus maculosus.* Waldo Shumway and James M. Sanders, University of Illinois. (10 min.)

The association of the thyreoid gland with amphibian metamorphosis has prompted attempts to induce metamorphosis in such forms as Necturus. Experiments conducted by the senior author have resulted so far only in the appearance of 'pseudometamorphosis' (Noble). A reexamination of the strictly morphological aspects of the development of this gland in Necturus has clarified the discrepancies to be found in the accounts of Platt and of Eycleshimer and resulted in the discovery of a median thyreoid in the full-grown Necturus, which, so far as we can learn, has not been described heretofore. The gland arises as a solid diverticulum from the pharynx, does not at any time acquire a lumen, grows caudad as far as the pericardium, splits into the two lateral and an anterior median bodies. Colloid formation is indicated at the 26-mm. stage. No budding of follicles, as has been described in Amblystoma, has been observed, and there is an actual decrease in the number of follicles between the 39-mm. stage and the full-grown condition. The median thyreoid is found ventral to the first basibranchial embedded in the connective tissue associated with the geniohyoid.

### *141. The effect of the removal of median and lateral areas of the anterior end of the urodelan neural plate on the development of the eye.* Howard B. Adelmann, Cornell University. (Lantern; 8 min.)

A median circular disc having a diameter about one-quarter to one-third of widest portion of neural plate was removed with the entomesoderm from the anterior end of the neural plate. Twenty-five of thirty-five Triton embryos possessed two essentially normal eyes, and four possessed two eyes, one of which was smaller than the other. Of forty-seven Amblystoma embryos, eleven were discarded as moribund; twenty-two possessed two essentially normal eyes; three, two eyes, one of which was smaller than the other; four, two small eyes, and five, degenerating or abnormal eyes.

Nine of twelve Amblystoma embryos from which a median strip of neural plate (width similar to, but length more than twice, that of disc) without entomesoderm had been removed, possessed two more or less typical eyes.

In three of four cases (Amblystoma) from which approximately a lateral third of the neural plate had been removed, the eye was represented on the operated side by a very small nodule, the size of which depended on area removed. One possessed two small nodules representing optic vesicles.

Of nineteen cases (Amblystoma) from which both the median and a lateral strip were removed, eleven developed from the remaining lateral strip a fairly typical eye; three, one small eye anlage, and three, two small eyes.

Camera-lucida drawings made immediately after operation allow correlation of operation with result.

Experiments indicate that removal of median strip does not prevent formation of two eyes. An eye may be formed when only one lateral third of the anterior end of the neural plate is left in situ.

### *142. The differentiation of median and lateral areas of the anterior end of the urodelan neural plate when implanted into the belly wall.* Howard B. Adelmann, Cornell University. (Lantern; 7 min.)

A median circular disc as described in the preceding abstract was removed from neural plate and implanted into the belly wall of Triton. In fourteen of twenty-eight cases implant differentiated into single eye. In the best cases this was beautifully differentiated; its relation to implanted brain was strikingly similar to that observed in cyclopean Amblystoma embryos. In six cases there was slight indication of doubling in implanted eye and in four cases two eyes were differentiated. In many cases surviving donors showed two typical eyes.

Strips of median third of anterior end of neural plate (Amblystoma) were transplanted with entomesoderm. About 50 per cent of implants which formed eye (about 64 per cent of total implants formed eye material) differentiated into two eyes more or less symmetrically related to implanted brain. When median strips were implanted without entomesoderm, about 50 per cent of implants which formed any eye material (about 63 per cent of total implants formed some eye material) differentiated into a single eye, although one implant with two eyes was found.

Strips of lateral third of neural plate (Amblystoma) were transplanted with entomesoderm; about 58 per cent of all implants formed tapetum and retina, although relations of these were sometimes atypical. When such strips were transplanted without entomesoderm, only about 13 per cent of all implants formed both tapetum and retina.

Complete analyses cannot be presented here. The experiments seem to indicate that, so far as neural plate alone is concerned, the eye-forming potencies are higher in median than in lateral portions. The entomesoderm seems to influence the bilaterality of the overlying plate and reinforce its eye-forming potencies.

### *143. The growth of the entire body and of the limbs of Amblystoma larvae during heteroplastic parabiosis.* ROBERT K. BURNS, JR., School of Medicine and Dentistry, University of Rochester. (Lantern; 15 min.)

Salamanders of the species Amblystoma punctatum and Amblystoma tigrinum differ greatly in capacity for total growth (size) and rate of growth and development. It was expected in heteroplastic parabiotic pairs that members of a pair would influence one another with respect to growth, particularly if the endocrines are fundamental regulators of these processes. Homoplastic pairs of A. tigrinum and normal larvae of A. tigrinum served as controls.

Food consumption as a factor in growth was controlled as far as possible by giving to members of pairs and to controls a fixed ration apportioned on the basis of the amount of food consumed ordinarily by a normal larva. The experiment covered most of the period of larval life. Under these conditions, the tigrinum member of heteroplastic pairs grew no faster than tigrinum members of homoplastic pairs or normal tigrinum larvae; nor did the limbs of such pairs exhibit either increased growth or precocious development. The tigrinum member of these pairs did develop abortive symptoms of a premature metamorphosis during the time when the punctatum member of the pair was metamorphosing. No heteroplastic pairs survived metamorphosis.

From this result it appears probable that, when diet is constant, capacity for growth in these animals is controlled by factors inherent in the tissues and is not susceptible of modification by the organic environment. Other interpretations are discussed.

### *144. Ultraviolet radiation as a means of twin production in Fundulus heteroclitus.* MARIE A. HINRICHS, University of Chicago. (Lantern; 10 min.)

In an earlier paper on monster production in Fundulus heteroclitus by means of ultraviolet radiation, it was reported that a certain small percentage of anterior twinning occurred when exposures were made early in the developmental period.

During the past summer attention was focused upon this early developmental period, i.e., all exposures were made at or before the first cleavage, with the view of producing a higher percentage of anterior twinning. With twenty-seven exposures made during the interval of from nine minutes to 116 minutes after fertilization, it was found that twelve exposures produced cases of anterior dichotomy. These twelve exposures were made during the period of from thirteen to thirty minutes after fertilization. The range of percentage of twin production varied from 0.2 to 12.5 per cent, with an average percentage of 6 per exposure.

The types of twins ranged from animals with two complete normal heads, and those with only one complete head and a second poorly developed head laterally or ventrally placed, to extremely inhibited forms in which only two separate axes, but little structural differentiation could be demonstrated.

### 145. *Concerning the etiology of congenital polydactylism in the descendants of x-rayed mice.* HALSEY J. BAGG, Memorial Hospital and Cornell University Medical College. (Lantern; 10 min.)

In 1926, Bagg demonstrated that certain forms of clubbing and syndactylism of the limbs of mice were associated with arrests in limb development and followed localized extravasation of blood. The extent of the pathological disturbance and the time of prenatal onset determined the type of morphological change. Thirty-two hundred animals had been examined, and sixty instances of polydactylism were noted. One polydactyl foot showed partial clubbing, one clubbing and syndactylism as well, and a third partial clubbing with a remnant of a blood-vascular lesion adjacent to the extra digit. It was suggested that in certain instances at least congenital polydactylism in this strain resulted from a localized arrest in embryonic development. At present 5595 animals have been examined (nineteen generations), and the following additional information is now available. In fourteen instances polydactyl feet have been found at birth showing the presence of partial clubbing in four instances; clubbing with the remnant of a localized hemorrhage adjacent to the extra digit in seven instances; and in three instances syndactylism of the toes adjacent to the extra digit. These results are based on the study of the first 150 animals to show polydactylism of one or more limbs. This type of abnormality has been found only in the hind limbs, ninety-seven right, sixty-five left; fifty-eight males, ninety-two females.

## *By demonstration*

### 146. *Insemination of starfish egg shown in motion pictures.* ROBERT CHAMBERS (assisted by C. G. Grand), Washington Square College, New York University.

Certain aspects of the peculiar insemination process in the starfish egg first hinted by Hermann Fol in 1879 and described by the writer in 1920 have been photographed in motion. There seems to be no doubt that the spermatozoon is dragged as a more or less passive body through the cortical 'jelly' to the surface of the egg by means of an ever-shortening filament which extends from the egg and is attached to the head of the spermatozoon.

147. *Microdissection of starfish eggs shown in motion pictures.* Robert Chambers (assisted by C. G. Grand), Washington Square College, New York University.

In motion pictures can be seen the destruction of the cytoplasm around the germinal vesicle of the starfish egg when the germinal vesicle is punctured with a micro needle. The formation and breakdown of surface films which impede the spread of the cytolysis is well known. In contrast to this is shown the impunity with which the cytoplasm can be punctured without undergoing cytolysis as long as the nucleus is not touched.

148. *Human embryo, 36 mm. (hysterectomy case), vascular system injected with India ink within three hours after removal from uterus.* Leon H. Strong, University of Michigan.

This embryo, cleared in benzylbenzoate, was fixed in 10 per cent formaldehyde, sectioned sagittally into right and left halves, and dissected grossly to show the body-wall drainage. This preparation demonstrated the arteries of the neck and thorax, the azygos and superior intercostal systems of veins, and the dorsal aortic plexus. On both sides the superior intercostal veins form anastomoses with the subclavian system of veins. The azygos vein joins the remnant of the dorsal aortic plexus in the first lumbar interspace and shows a segment of less than one interspace contributed by this plexus.

149. *Variations in mouse embryos of eight days' gestation.* Ezra Allen and E. C. MacDowell, Carnegie Institution of Washington.

The demonstration consists of embryos and photographs showing variations in size and development in the ovum at the end of the first day of the embryo proper. Variation between litters is considerable. In the same litter embryos may be at nearly the same stage of development or vary from the presomite stage to seven somites. Litter variation is independent of litter size. Variation within the litter is independent of litter size, of number in one uterine horn, and of position in horn.

150. *Maturation in the parthenogenetic and sexual eggs of Moina macrocpa, a cladoceran.* Ezra Allen, Carnegie Institution of Washington.

The chief interest in maturation in this form is that apparently sex control is established about four hours before maturation. There is only one maturation division in the parthenogenetic, and apparently only one in the sexual, egg. No reduction occurs in the parthenogenetic egg. In the sexual egg the chromosome number is reduced from 22 to 11. The number in the female-producing parthenogenetic egg is 22. The number in the male-producing egg has not been exactly determined, but is more than 16, so is not haploid. A large amount of nucleolar substance, between the several variously shaped and sized bodies of which lies achromatin, characterizes the nucleus until the egg is nearly ripe. These two substances conceal the true chromatin up to this stage. With the degeneration of the nucleolar substance, which seems to function as nutritive material for the ooplasm, true chromatin appears, a spindle forms, and just before the egg is laid the chromosomes appear in late prophase or metaphase near the periphery of the egg. Maturation division occurs in the parthenogenetic egg within three to five minutes after the eggs are laid; in the sexual egg, much later. The cytologic conditions within the egg at the period when sex control occurs have not been determined.

*151. Parthenogenesis in lampreys; ova in male gonads of the brook and of the lake lamprey.* Simon H. Gage, Cornell University.

Demonstration of lamprey eggs developing to the morula stage without fertilization. Microscopic specimens of the eggs showing the blastocoele and mitotic figures in dividing egg cells. Photographs and microscopic specimens of the ova in the male gonad.

*By title*

*152. Germ-cell origin in the lake lamprey (Petromyzon marinus unicolor).* E. O. Butcher, Cornell University. (Introduced by H. D. Reed.)

An effort has been made in this study to determine whether the germ cells of the lake lamprey are segregated early or arise from peritoneal epithelial cells.

Large yolk-laden cells which have generally been called primordial germ cells were found in the caudal end of the embryo just before the appearance of a distinct entoderm. A greater part of these soon degenerate; however, some persist and apparently become functional sex cells. The definitive germ cells are derived, for the most part, from the coelomic epithelium. Proliferation and successive transitional stages were found frequently in larvae of 7 to 18 mm. in length. The potentiality of the epithelium is gradually lost after the formation of the gonad.

Since the yolk-laden cells cannot be traced back to cleavage stages and germ cells may arise from the epithelium, there is no evidence of a distinct germ track. Three suggestions are given as explanation for the presence of the yolk-laden cells: 1) They may represent germ-cell formation as a result of cellular differentiation in very early stages; 2) they may be a vestige of the segregated germ cells of lower organisms, while the transformed peritoneal cells are representative of the condition found in mammals where germ cells arise from the germinal epithelium; 3) they may act as a stimulating factor to the formation of a gonad, and germ cells from the epithelium.

*153. Origin of the germ cells in Amblystoma maculatum.* Gladys K. McCosh, Cornell University. (Introduced by the Secretary of Section F.)

This study is based upon a close series of ontogenetic stages from cleavage until metamorphosis. The so-called primordial germ cells, first differentiated in the lateral mesoderm, are traced to a definitive position in the genital anlagen. Careful counts of these demonstrated that they exist in larger numbers in the younger stages and that few of them ever reach the genital anlagen where they may form a small portion of the propagative cells.

Evidence is presented that the majority of the germ cells are of somatic origin. From the earliest appearance of the indifferent gonad certain cells in the germinal epithelium have been observed which were increasing markedly in size and undergoing the various changes necessary in the transformation of small, cuboidal, or spindle-shaped peritoneal cells with oval nuclei into large germ cells with immense polymorphic or lobate nuclei. These cells are abundant in all older individuals. All the successive stages in the evolution of a somatic cell into a reproductive cell, involving as it does an increase in size, changes in shape, and a new distribution of chromatin material, are demonstrated.

### 154. *The development of fertilized fragments of the annelid egg.* Edmund B. Wilson, Columbia University.

It was observed by Delage ('99) in Lanice and more adequately by F. R. Lillie ('09) in Chaetopterus that fertilized fragments of the annelid egg typically divide into two unequal cells showing the same size ration as in the division of whole eggs. Delage also figured a normal four-cell stage (confirmed in Lanice by the writer) and a dwarf trochophore, both from egg fragments. The writer has found that in Chaetopterus the fertilized fragments often undergo complete cleavage like that of whole eggs, showing the typical cell proportions and alternation of spiral cleavages. If not too small, they commonly develop into active dwarf larvae, often abnormal (though usually bearing a typical apical flagellum), but sometimes closely approaching the normal in form, structure, and swimming movements. Nearly normal dwarfs may arise from hyaline fragments almost devoid of granules.

It is not yet known whether fragments from all regions of the egg are capable of producing normal dwarfs, as seems to be the case in the nemertine (Wilson, '03). Doubt in regard to this arises from the presence of a distinct lower polar area in the oocyte (Lillie), as in Dentalium (Wilson, '04); also by the fact that, as in Dentalium, the first cleavage of the fragments may or may not take place with a 'polar lobe.' A study of fragments of fertilized eggs at successive periods, such as that made by Yatsu and by Zeleny in the nemertine egg, should throw light on this question.

### 155. *The effect of ultraviolet radiation on cleavage in the egg of Arbacia.* R. A. Young, Howard University. (Introduced by E. E. Just.)

Experiments were made at the Marine Biological Laboratory, Woods Hole, Massachusetts, during 1927 and 1928, on eggs of Arbacia which were exposed immediately before insemination to ultraviolet for lengths of time varying from thirty to 180 seconds. The results show retardation and abnormalities in cleavage which increase directly with length of exposure.

Study of the living and sectioned egg and experiments on fertilized eggs reveal that the retardation of cleavage is due to a prolongation of the 'pause' stage in mitosis. No other mitotic stage is affected. The first cleavage spindle is markedly eccentric. The abnormalities observed are: 1) abnormal behavior of the cortex; 2) aggregation of pigment to form a disc at the cortex in the plane of first cleavage; 3) unequal cleavage, and, 4) partial cleavage. These effects are wholly cortical reactions to radiation and are sufficient to explain the modifications observed in later developmental stages.

### 156. *Development of the germ band and the embryonic envelopes of Diacrisia virginica Fabr.* O. A. Johannsen, Cornell University.

The cells destined to form the serosa reach the periphery first, are two- to four-nucleate, and larger than the uninucleate cells of the embryonic rudiment. The embryonic rudiment, at first band-like, encircles the yolk at the equator of the egg. The serosa, the part not covered by the embryo, soon overlaps the latter, growing over it. The amnion does not begin to form until the serosa wholly covers the surface. At eighty-four hours, a fold of the amnion grows over the dorsal side of the embryo, within a few hours entirely closing it. A small portion of the amnion thus forms the dorsal wall of the embryo, while the greater part forms an embryonic envelope. A second dorsal organ is not developed. With the completion of the amnion (in about eighty-seven hours) the embryo, which thus far lies curled up, with the ventral side directed outwardly, now rolls over on its longitudinal axis so as to lie with the dorsal side outmost. Such yolk as still remains outside of the gut at the time of closure of the dorsal umbilicus is consumed by the larva before its emergence, which takes place at about six and one-half days. The amnion ruptures just before the larva begins feeding on the yolk; the serosa ruptures at the time of hatching.

*157. Notes on a series of late embryos and newly hatched young of the chimeroid Callorhynchus.* L. HUSSAKOF, American Museum of Natural History.

The object of this study was to determine the size at which Callorhynchus hatches from the egg case and the morphological changes, if any, it undergoes from just before hatching until it attains adult form. The material is a well-graded series of seven specimens, six in the British Museum (of which I made careful drawings when there), and one, a fine specimen of a late embryo in the egg case, in the American Museum.

The earliest stage in the series is a late embryo 122 mm., with a yolk sac 40 mm. in length, which had been removed from its egg case (Brit. Mus.). The next is an embryo 130 mm., with a yolk sac 19 mm. The third is a newly hatched young, 136 mm. in length, showing only a seam where the yolk sac had been. Hence hatching occurs at a length of about 135 mm. The other young in the series are 160, 221, and 240 mm. The young at hatching shows all the characters of the adult in fully developed form, including the details of the lateral-line system of the head.

## PARASITOLOGY

### *Read*

*158. A quantitative study of fowl coccidiosis.* BENJAMIN P. YOUNG, Cornell University. (Lantern; 15 min.)

A lantern slide will be shown indicating graphically the results of three consecutive inoculations of a fowl with sporulated oocysts of Eimeria avium. The number of oocysts appearing daily in the faeces is taken as an index of the course of the disease. From the curve both the prepatent and patent periods of fowl coccidiosis may be seen at a glance. Conclusions may also be drawn as to whether the experimental inoculation of chicks with coccidial oocysts will result in an immunity or a high resistance to coccidiosis. The relation of coccidiosis to fowl paralysis, as indicated by this work, will be pointed out.

*159. A microscopical study of glochidial immunity.* LESLIE B. AREY, Northwestern University Medical School. (Lantern; 15 min.)

Glochidial cysts on the gills of immune fishes form in the same manner as normal ones, but they tend to grow larger and become more irregular. The increased thickness is due to additional cellular connective tissue in the wall.

The gill tissue indicates the existing biological incompatibility only by the presence of eosinophiles, extruded chromatin spherules, and eosinophilic plastids.

In natural, or racial, immunity many glochidia are promptly destroyed by cytolysis, accompanied by an invasion of host cells. These disintegrating glochidia may occur in close proximity to unaffected glochidia, and apparently are merely less resistant individuals that succumb to a critically adjusted reaction.

In both natural and acquired immunity the normal retention of glochidia and the accompanying metamorphosis is replaced by premature shedding. After the first day the cyst thins by the removal of stroma cells back into the filament until the wall is reduced to a thin envelope. Both intact and destroyed glochidia, and apparently their cyst coverings, are sloughed at about the second day. Repair of the resultant notched filament is prompt.

### *By demonstration*

### 160. *The life-history of Cryptocotyle lingua (Creplin).* Horace W. Stunkard, New York University.

Ryder ('84) and Linton ('00) reported metacercariae, encysted in the skins, fins, and gills of Tautogolabrus adspersus and other marine fishes from the north Atlantic coast. Linton ('14) showed that these encysted larvae comprise one stage in the life-cycle of Cryptocotyle lingua. According to Ciurea ('24), the American species of Cryptocotyle, which he named C. americana, is not identical with C. lingua of Europe. During the summer of 1928 the parthenogenetic stages of this species were identified and the life-history demonstrated by developing the larvae in fish and in the cat, rat, and guinea-pig. The cercariae occur at Woods Hole in the digestive gland of Littorina littorea. They are produced in elongate, sac-shaped rediae which have the flame-cell formula $2x\ (3+3)$. Liberated cercariae attacked T. adspersus, boring into the skin in enormous numbers. The encysted specimens were fed to experimental animals and developed into adults, identical with those found in natural infestations. The cercaria was found by Lebour in the liver of L. littorea and Paludestrina stagnalis on the British coast. Since L. littorea is a European snail, introduced on the American shore during the past century, and since it harbors the same cercaria on both sides of the Atlantic, presumably the species of Cryptocotyle found in America is identical with C. lingua. Lebour ('12) tentatively assigned the cercaria from L. littorea to Cercaria lophocerca Filippi 1857, but the description of that species is too brief to permit a final determination.

### *By title*

### 161. *The specificity of metazoan parasites for the host in its relation to the phenomena of age resistance and acquired immunity.* J. H. Sandground, Harvard Medical School.

Other than natural immunity, which is essentially due to an incompatibility between the species of host and parasite involved, two distinct types of resistance to infection are recognized, namely, acquired immunity, which is specific and is a consequence of previous infection of the host, and so-called 'age resistance,' by virtue of which increasing age confers an immunity against infections to which the host is more or less susceptible when young. Several instances of both phenomena have now been demonstrated to occur for metazoan infections. Age resistance does not apply to a single species of parasite, whereas the host's acquired immunity is specific for the parasite which induced the immunity. Analysis of the situation shows that the majority of instances of age resistance definitely involve species of parasites which are abnormal in the host, and although many of the species of parasites which have been shown to incite immunity (acquired) involve an abnormal host, this host reaction may also be exhibited against a normal parasite.

Consideration of the nature of specificity indicates that it is a reflection of the extent to which the parasite is adapted to the host and is a product of an evolutionary process. This view provides a basis for an understanding of the nature of age resistance. A consideration of the specificity in the host-parasite association may also afford a clue for investigating the defense mechanism underlying acquired immunity in certain infections.

### *162. An acanthocephalid, Corynosoma strumosum (Rudolphi), in the California harbor seal.* GORDON H. BALL, University of California at Los Angeles.

A very young specimen of the California harbor seal, Phoca richardii, recently taken off Point Mugu, Ventura County, California, was found to be heavily infected with specimens of the acanthocephalid, Corynosoma strumosum, some of which had reached sexual maturity. Since the young seal was only about 3 feet long and still had the umbilical cord present, it is believed that the animal could not have been over three weeks old. A great number of small worms were found attached to the lining of the anterior portion of the small intestine and others were lying free in the lumen. By actual count, 1154 parasites were removed; at least 5 per cent more were unavoidably lost in the food mass. The captured animal was obviously weak and ill; since no evidence of other infection was visible macroscopically, it is probable that the symptoms were due to the exceptionally heavy degree of parasitism. This is believed to be the first recorded case of an infection of the California harbor seal by an acanthocephalid. The presence of ripe eggs in some of the parasites indicates that a period of about three weeks or less is necessary for Corynosoma to reach maturity in the seal.

## ECOLOGY

### *Read*

### *163. The distribution of the Cyprinidae in the Great Lake Region.* T. L. HANKINSON, Michigan State Normal College. (Introduced by the Secretary of Section F.) (12 min.)

From data obtained through collecting fish and making field observations and from studying card records accompanying the fish collections at the Museum of Zoology of the University of Michigan and from examination of literature, inferences are made as to the character of the habitats of the different species of minnows found in the Great Lake drainage.

Some species live under a great variety of conditions having a wide distribution in the region. Other species are more restricted in distribution, occurring in or about dense growths of aquatic plants, while others avoid such situations and dwell more in the open where plant growths are absent or scant. There are a number of minnows that appear to be strictly stream forms and are unusual elsewhere, excepting one which is often abundant in Great Lake waters that have frequent wind disturbance. It was found that the young and adults of many of the minnows have very different habitats; the former often congregate in marginal, sunlit shallows away from the adults in deeper water. Correlations have been noted between the occurrence of certain cyprinids and depth and temperature of water, bottom features, especially those favoring spawning, extent of habitat conditions, and presence or absence of organisms that affect the minnows favorably or unfavorably. An important result of the work is the accumulation of facts pertaining to the little-understood economic status of the different species of Cyprinidae in the Great Lake Region.

*164. The distribution of varieties and species of Partula upon the islands of Tahiti and Raiatea.* W. H. LONGLEY, Goucher College. (Lantern; 12 min.)

When the distribution of the varieties of Partula otaheitana and P. clara is plotted, patterns essentially similar are obtained which have an axis of symmetry coinciding with that of the prevailing wind, the southeast trades. It is a natural inference that local differences in humidity, determined by the shape and size of the island and the direction of the on-shore wind, have controlled the development and fixed the ranges of new forms within these two species upon parallel lines.

Two checks upon this conclusion are possible. First, from their behavior upon Tahiti nui, certain conclusions may be drawn regarding the qualities of different varieties, and these conclusions may be verified from observation of their distribution upon the peninsula of Taiarapu, a land mass of much the same shape, but smaller size, than the main island. Secondly, Garrett's data upon the distribution of Partula species upon Raiatea show that when due allowance is made for its different shape its species in their plotted ranges repeat the pattern shown upon Tahiti.

The facts constitute a very interesting commentary upon the closeness of the correlation between specific aptitudes and habitats, which it scarcely seems possible to explain without appeal to natural selection.

*165. The work of the University of Montana Biological Station at Flathead Lake.* M. J. ELROD AND R. T. YOUNG, University of Montana. (Lantern; 15 min.)

A general description of Flathead Lake, its physics, chemistry, and biology, with especial reference to the fish of the lake, their ecological relations, and the introduction of the Great Lakes whitefish (Coregonus clupeiformus).

*166. A biological study of the Big Muddy River and its tributaries, polluted by domestic sewage and industrial wastes.* H. P. K. AGERSBORG, State of Illinois, Department of Conservation. (8 min.)

In the middle of summer, owing to extensive deforestation along its course, the Big Muddy River is in some places only a series of pools or a hardly flowing shallow stream. Formerly it was navigable for many miles. Up to thirty years ago, it was noted for its importance for fishing. It was the best fishing station in southern Illinois. At the present time the river is not only irregular in its periodic volume, but it is highly turbid and pretty badly polluted with mine water, creosote-, dairy-, gas-plant, packing-house wastes and domestic sewage. Annually the Big Muddy River is 'flushed' by backwater from the Mississippi River and then automatically restocked with fish. But during low water, when the tributaries are the most highly polluted, a local shower over one of them will, when carried down into the main stream, stun and finally kill off every fish from the mouth of the tributary and down for many miles. In practically all of the tributaries, all fish, mollusca, and crustacea are extinct. In the Big Muddy itself the mollusks are destroyed, save for a stretch of about 600 feet in the middle of the stream at Blairsville. Nearly all birds and game along the banks are extinct. But indications are that if pollution were abrogated the fish, birds, and game would return and the river basin would become an attractive playground for southern Illinois. The industries, operating to-day, might operate then as well.

167. *Observations on certain littoral decapod crustaceans, with special reference to migrations from marine to terrestrial habitats.* A. S. PEARSE, Duke University. (Lantern; 10 min.)

Hermit crabs which have become more or less adjusted to life on land show a progressive reduction in the number of gills, and Cenobita diogenes lives after its gills have been completely removed. Crabs which have migrated landward, in general, have progressively smaller gills, and often have a reduction in number. Beach animals which show any landward trend usually live longer when kept in air than when kept in fresh water. Animals which have attained some degree of ability to live on land have often also acquired a greater degree of ability to resist the extraction of constituents of body fluids into fresh water. Segregation into particular habitats is apparently an important factor in the migration of marine animals to land. An ideal land animal is air-breathing, water-conserving, swift, and internally stable. Crabs, which live more or less continually on land, have parasitic mites in their gill chambers, but those which frequently visit the ocean contain none.

### *By demonstration*

168. *Factors determining the attachment of the cyprid larvae of the barnacle, Balanus galeatus.* J. PAUL VISSCHER, Western Reserve University.

The barnacle, Balanus galeatus, attaches only on certain Alcyonarian corals (Leptogorgonia). The cyprid larvae, however, cannot attach directly to this coelenterate, as the polyps effectively feed upon all such organisms within their range. Other forms in addition to this barnacle are found living upon these corals. A nudibranch, a snail, and a shrimp are all associated, apparently also living only on these corals. The egg cases of both the nudibranch and the snail are fastened as densely spiraled masses near the growing tips of the coral. The jelly mass containing the eggs apparently exerts a chemical attraction upon the cyprid larvae of this barnacle which penetrate it and metamorphose in contact with the coral, while the surrounding polyps are held harmless by virtue of the gelatinous mass surrounding them. By the time this mass has disintegrated, the barnacle is well established and attached directly to the horny axis of the coral.

169. *On the formation of saliniclines in Barnegat Bay, New Jersey, with observations of the reactions of oyster larvae to salinity gradients.* T. C. NELSON, Rutgers University.

Owing to the small tidal flow (10 to 15 cm.) and to the comparatively level contour of the bottom of Barnegat Bay, New Jersey, the heavier sea-water coming in through the inlet with the flood tide remains close to the bottom, the waters of lower salinity lying above. Over most of the deeper regions, 2 to 3 meters, of this estuary the layer of high-density water seldom rises above 1 meter. The transition zone, salinicline, between the lower water of high density and the upper water of low density is usually abrupt. In this zone an increase in specific gravity from 1.01000 to 1.01700 (S.13 to 22.3) within 0.3 meter has been observed. Such stratification occurs only during relatively calm weather, since wave action with northerly or southerly winds in excess of twenty-five miles reaches to the bottom. Salinicline formation is most pronounced when flood tide occurs in the early morning and with the higher tides. The transition zone forms a shearing plane where the overlying waters slip over the lower layers which may show little or no movement. The flow here may even be in the opposite direction to the movement of the upper layers. Oyster larvae show a sharp reaction to the salinity gradient, being found by thousands at the top of the salinicline, while the lower waters contain few or none.

*By title*

*170. The distribution and time of breeding of shrimps in Chesapeake Bay.* R. P. COWLES, Johns Hopkins University.

The following conclusions concerning the distribution and breeding season of shrimps in Chesapeake Bay are based on some data from the U. S. National Museum and on collections made for the U. S. Bureau of Fisheries at rather definite intervals through the year. About thirty stations widely distributed through the offshore waters of the bay have been visited. A large tow net attached to a beam trawl and drawn along the bottom, another tow net drawn vertically from the bottom to the surface, and smaller surface tow nets have been used in this work.

Of the three species occurring commonly, Palaemonetes carolinus frequents shallow water usually near the tide lines. Only a few specimens (taken exclusively during the winter and early spring) have been found on our cruises, although they are known to occur in considerable numbers along the shore line. Ovigerous specimens have been caught in July.

Palaemonetes vulgaris seems to have a similar distribution, but it is found more frequently in somewhat deeper water, judging from the results of our towings. Ovigerous specimens and larvae have been caught during the summer months.

Crangon septemspinous is a shrimp of the deeper waters. It has been taken frequently throughout the year over the whole bay, although it has been captured in largest numbers during the fall, winter, and spring. The records of Schmitt show that juvenile individuals occur at all seasons.

*171. Concerning the ecology of the muscid fly, Pyrellia serena Meigen, with special reference to hibernation.* A. M. HOLMQUIST, St. Olaf College. (Introduced by W. C. Allee.)

During field studies on hibernating arthropods, Pyrellia serena was found to be one of the minority having gregarious overwintering habits. Observations and experiments have been made on this account and because the life-history is not recorded. The species is abundant in open woods near Chicago and occurs also at Northfield, Minnesota. Hibernation extends from November to early April. The flies hibernate in old insect burrows and under loose bark of large logs and stumps in mid or late decay. They may hibernate singly, but more often are in small groups which may be of one or both sexes. In these aggregations the flies are all in the same plane clinging to the sides of the cavity, usually contiguous. They are not scattered throughout all available spaces as are solitary insects. Pyrellia may form accidental heterotypic aggregations with other insects, including predaceous forms.

The flies maintain enough activity to crawl slowly to cover when exposed. Unless strongly stimulated, they are photonegative in winter. Hibernating flies brought into the laboratory responded to warming, but never showed the activity of summer. They fed on condensed milk and somewhat on sugar solutions. When fed, they lived about forty days at room temperature, but could not be induced to breed. Typical hibernation aggregations were produced in the laboratory by lowering the temperature gradually. I have little information concerning summer activity or habitat. None was found on the wing until late fall when nearly ready to hibernate.

### *172. The effect of climatic and weather factors upon the number of birds on a depositing creek bank near Nashville, Tennessee.* JESSE M. SHAVER, George Peabody College for Teachers. (Introduced by W. C. Allee.)

The field work involved eighty-two trips distributed over eighty-two weeks. The numbers of birds were smoothed and compared with six smoothed weather factors (which approximate climate). Permanent residents and winter visitants are the most conservative. In general, for all groups, the coefficients of correlation rank in this order: 1) Temperature; 2) light (plus in fall and winter and minus in spring and summer); 3) relative humidity (minus in winter and plus in summer); 4) wind velocity; 5) atmospheric pressure; 6) precipitation.

The deviations of the different bird groups and the deviations of the weather factors were also correlated. For the most part, these correlations are low with rather high probable errors, and hence cannot be regarded as of the same significance as many of those with climatic factors. Here, as with climate, permanent residents and winter visitants are most conservative. Summer residents show the highest correlations and in the following order: 1) Sunshine; 2) relative humidity (minus correlation); 3) wind velocity; 4) atmospheric pressure; 5) temperature.

### *173. Transformation sizes of the Ranidae of the United States and Canada.* A. H. WRIGHT, Cornell University.

In working on the life-histories of North American Salientia north of the Mexican line we have considered transformation (sizes, periods, and other factors) along with other topics. Herein we present only the sizes at transformation of the Ranas of the United States and Canada: 16–18-mm. Rana sylvatica, Rana cantabrigensis; 16–23-mm. Rana pretiosa; 19–27-mm. Rana pipiens; 20–33-mm. Rana sphenocephala; 21–26-mm. Rana boylii sierrae; –29-mm. Rana boylii muscosa; 22–30-mm. Rana boylii boylii; 22–27-mm. Rana aurora draytoni; – – –Rana aurora aurora; 23–30-mm. Rana virgatipes; 25.5–38-mm. Rana clamitans; 28–38-mm. Rana aesopus; – –30-mm. Rana areolata; 29–38-mm. Rana septentrionalis; at least below 39-mm. Rana onca; 30–39-mm. Rana heckscheri; 32–49-mm. Rana grylio; 43–49-mm. Rana catesbeiana.

# PROGRAM AND ABSTRACTS FOR THE JOINT GENETICS SECTIONS OF THE AMERICAN SOCIETY OF ZOÖLOGISTS AND THE BOTANICAL SOCIETY OF AMERICA

Chairman, H. J. MULLER, University of Texas, Austin, Texas
Secretary, L. C. DUNN, Columbia University, New York

## PROGRAM OF THE EIGHTH ANNUAL MEETING, DECEMBER 27–29, 1928

### THURSDAY MORNING SESSION, DECEMBER 27TH, 10.00 A.M.

Room 304, Dodge Hall, Teachers College

1. An unexpected association of factors belonging to three linkage groups in Oenothera and its explanation. George H. Shull, Princeton University.
2. A case of doubling of chromosomes in a cross between Nicotiana rustica and N. paniculata. W. R. Singleton, Connecticut Agricultural Experiment Station. (Introduced by E. M. East.) (10 min.)
3. New evidence bearing upon the problem of the cytological basis for genetical peculiarities in the oenotheras. R. E. Cleland and Fr. Oehlkers, Goucher College. (15 min.)
4. The inheritance of semisterility in maize. R. A. Brink and C. R. Burnham, University of Wisconsin. (Lantern; 10 min.)
5. A haploid mutant in the tomato. E. W. Lindstrom, Iowa State College. (Lantern; 8 min.)
6. The operation of inhibiting factors in the determination of fruit shape in the squash. E. W. Sinnott, Columbia University. (Lantern; 8 min.)
7. A lethal factor in Crepis effective only in an interspecific hybrid. Lillian Hollingshead, University of California. (Introduced by E. B. Babcock.) (15 min.)
8. The occurrence of chromosomal variants in Nicotiana alata. T. H. Goodspeed and Priscilla Avery, University of California. (Lantern; 10 min.)
9. The frequency of somatic mutation in variegated pericarp in crosses of variegated with colorless maize. R. A. Emerson, Cornell University. (Charts; 15 min.)

*To be presented if time permits*

10. Linkage between quantitative and qualitative genes in maize. I. Number of rows and cob color. E. W. Lindstrom, Iowa State College. (Lantern; 12 min.)

The following papers may be called up at the end of the session if time remains: No. 25 (Randolph) and no. 58 (Abegg).

### THURSDAY AFTERNOON SESSION, DECEMBER 27TH, 2.00 P.M.

Room 301, Dodge Hall, Teachers College

Joint session with Geneticists Interested in Agriculture.

1. Symposium: Inheritance of disease resistance in plants and animals. Leaders: (from the botanical standpoint) H. K. Hayes, University of Minnesota; (from the zoölogical standpoint) L. J. Cole, University of Wisconsin.

The following exhibits and demonstrations will be shown in the exhibit room (305 Dodge Hall) after the joint session: Nos. 28, 31, 36, 38. It is hoped that most of the other exhibits will also be on view by 4 o'clock on Thursday.

### FRIDAY MORNING SESSION, DECEMBER 28TH, 10.00 A.M.

Room 304, Dodge Hall, Teachers College

11. Some physicochemical aspects of life, mutation, and evolution. Jerome Alexander, New York City, and C. B. Bridges, California Institute of Technology. (15 min.)
12. The rate of induced mutation in relation to dormancy, temperature, and dosage. L. J. Stadler, University of Missouri. (Lantern; 15 min.)
13. Radium experiments with Datura. I. The identification and transmission of lethals of pollen-tube growth in $F_1$'s from radium-treated parents. J. T. Buchholz and A. F. Blakeslee, University of Texas and Carnegie Institution, Cold Spring Harbor, New York. (15 min.)
14. Radium experiments with Datura. II. Pollen abortion in $F_1$'s from radium-treated parents. J. Lincoln Cartledge and A. F. Blakeslee, University of Pittsburgh and Carnegie Institution, Cold Spring Harbor, New York. (7 min.)
15. Radium experiments with Datura. III. Chromosomal abnormalities in $F_1$'s from radium-treated parents. A. Dorothy Bergner, Sophia Satina, and A. F. Blakeslee, Carnegie Institution, Cold Spring Harbor, New York. (15 min.)
16. Radium experiments with Datura. IV. Visible gene mutations in radium-treated and control material. A. G. Avery and A. F. Blakeslee, Carnegie Institution, Cold Spring Harbor, New York. (7 min.)

(Radium experiments with Datura. V. J. T. Buchholz and A. F. Blakeslee, by title and demonstration, see paper 23.)

17. Visible mutations from radium rays. Elvene Winkleman, Washington University. (Introduced by Caswell Grave.) (10 min.)
18. An analysis of the effects of the different rays of radium in producing lethal mutations in Drosophila. Frank Blair Hanson, Washington University. (15 min.)
19. Chromosome translocations produced by x-rays in Drosophila. H. J. Muller, University of Texas, and Edgar Altenburg, Rice Institute. (15 min.)

*To be presented if time permits*

20. Genetic effects of x-rays and other environmental agents. Alexander Weinstein, University of Minnesota. (15 min.)

Following the papers there will be a Business Meeting of the Genetics Sections (to begin about 12.15).

### FRIDAY AFTERNOON SESSION, DECEMBER 28TH, 2.00 P.M.

Rooms 304 and 305, Dodge Hall, Teachers College

*Demonstration papers*

The demonstration papers have been arranged in four groups, and each group has been assigned to a one-hour period. The authors of papers assigned to each period will be present at their exhibit during that hour to explain their results or give their demonstrations. At other times the exhibit will be visible, but it is not expected that the author will be in attendance unless special arrangement is made.

In addition to the demonstrations listed below, material illustrating papers nos. 4 (Brink); 7 (Hollingshead); 8 (Goodspeed and Avery); 45 (Holmes); 58 (Abegg); 60 (Hunt and Permar), and probably others will be shown in the exhibit room.

*Demonstrations group I. 2.00 to 3.00 P.M.*

21. The comparative effects of the same genes in different quantities. Robert K. Nabours, Kansas State Agricultural College. (Charts; 10 min.)
22. Wing production in aphids as affected by light and temperature. A. Franklin Shull, University of Michigan. (Experimental apparatus and microscopic preparations.)
23. Radium experiments with Datura. V. Effects of radium on pollen-tube growth and the seed output following treatment. J. T. Buchholz and A. F. Blakeslee, University of Texas and Carnegie Institution, Cold Spring Harbor, New York.
24. A recessive factor for polydactylism in man. L. H. Snyder, North Carolina State College. (Charts.)
25. Types of supernumerary chromosomes in maize. L. F. Randolph, Cornell University.

*Demonstrations group II. 3.00 to 4.00 P.M.*

26. Studies on pigeon hybrids. II. The chromosome conditions as an index of differential mortality of embryos. L. J. Cole, T. S. Painter, and Agnes Zeimet, University of Wisconsin and University of Texas. (Microscopic preparations.)
27. Chromosome morphology in nine- and ten-paired species of Nicotiana. Priscilla Avery, Lillian Hollingshead, and Lillian J. Raup, University of California. (Microscopic preparations.)
28. Linkage of the characters albinism and shaker in the house mouse. William H. Gates, Louisiana State University.

29. A comparison of the effects of extra chromosomes on pollen-tube growth from pollen of various (2n + 1) types of Datura stramonium. J. T. Buchholz and A. F. Blakeslee, University of Texas and Carnegie Institution.
30. Hybrid vigor in poultry. D. C. Warren, Kansas State Agricultural College. (Charts.)
31. Innate limitations of growth rate in the mouse. E. C. MacDowell, W. H. Gates, and C. G. MacDowell, Department of Genetics, Carnegie Institution of Washington, Cold Spring Harbor, Long Island, New York, and Louisiana State University. (Charts.)

*Demonstrations group III. 4.00 to 5.00 P.M.*

32. Heredity of miniature (body), an x-ray mutation in Habrobracon. P. W. Whiting, University of Pittsburgh. (Specimens and charts.)
33. Integumental grafting of the Leghorn fowl. A. W. Kozelka, University of Pittsburgh. (Living specimens.)
34. A third linkage group in the killifish, Platypoecilus. Allen C. Fraser and Myron Gordon, Cornell University. (Living specimens.)
35. A survey of flower colors in the garden balsam and of the genes affecting them. Donald W. Davis and Lucy Ann Taylor, College of William and Mary.
36. A new type of congenital hypodactylism of the right limbs occurring in the sixteenth to nineteenth generations of the descendants of x-rayed mice. Halsey J. Bagg, Cornell University Medical College and Memorial Hospital, New York.

*Demonstrations group IV. 5.00 to 5.30 P.M.*

37. On the several generations of daughters from repeated matings of daughter to sire. H. D. Goodale, Mt. Hope Farm, Williamstown, Massachusetts. (Charts.)
38. Living mice and museum specimens showing congenital morphological abnormalities of the viscera, eyes, and limbs. Halsey J. Bagg, Cornell University Medical College and Memorial Hospital, New York.
39. Identity of genes and sex factors in intergeneric killifish hybrids. Allen C. Fraser and Myron Gordon, Cornell University.

### SATURDAY MORNING SESSION, DECEMBER 29TH, 10.00 A.M.

Room 304, Dodge Hall, Teachers College

40. Multiple fission in Heteronema acus. Robert C. Rhodes, Emory University. (Lantern; 15 min.)
41. Heritability of the effects of ultraviolet and infrared waves on fission rate within a clone of Paramecium caudatum. Austin R. Middleton, University of Louisville. (Lantern; 10 min.)
42. Four-strand crossing over. Alexander Weinstein, University of Minnesota. (15 min.)
43. Eye defects in rabbits. Heman L. Ibsen and L. D. Bushnell, Kansas State Agricultural College. (Lantern; 15 min.)
44. Twinning in some fresh-water snails. Edward D. Crabb, University of Pennsylvania. (Lantern; 10 min.)

45. The determination of identity in twins. S. J. Holmes, University of California. (Charts; 10 min.)
46. Sex determination and sex-ratio determination in Sciara. Charles W. Metz, Department of Genetics, Carnegie Institution of Washington, Cold Spring Harbor, New York. (15 min.)
47. The establishment of thyroid races in pigeons. Oscar Riddle, Department of Genetics, Carnegie Institution of Washington, Cold Spring Harbor, New York. (Lantern; 15 min.)
48. Studies on pigeon hybrids. I. Aberrant sex ratios. L. J. Cole, T. S. Painter, and Agnes Zeimet, University of Wisconsin and University of Texas. (Lantern; 15 min.)
49. The critical period for sex control and the possible mechanism of sex determination in Cladocera. A. M. Banta and L. A. Brown, Department of Genetics, Carnegie Institution of Washington, Cold Spring Harbor, New York, and State University of Iowa. (Lantern; 15 min.)

*To be presented if time permits*

50. The functional relation of the genes in the X-chromosome of Drosophila melanogaster referred to bar eye as base. A. H. Hersh, Western Reserve University. (Lantern; 10 min.)
51. The inheritance of egg weight in domestic fowl. F. A. Hays, Massachusetts Agricultural College. (Lantern; 15 min.)

*Papers to be read by title*

52. The effect of long subjection to constant temperatures upon the bar eye of Drosophila melanogaster. Charles Zeleny, University of Illinois.
53. The temperature coefficient and temperature-effective period for wing size in Drosophila. Willard F. Stanley, University of Illinois. (Introduced by Charles Zeleny.)
54. The effect of temperature on infrabar and its heterozygotes. W. M. Luce, University of Illinois. (Introduced by Charles Zeleny.)
55. The occurrence of parthenogenesis in the genus Chrysanthemum. E. C. Jeffrey, Harvard University.
56. The inheritance of reproductive characteristics in Daphnia longispina. Kathryn Ordway, Department of Genetics, Carnegie Institution, Cold Spring Harbor, New York. (Introduced by A. M. Banta.)
57. Inheritance in Mongolian idiocy. Madge Thurlow Macklin, University of Western Ontario.
58. Some effects of the waxy gene in maize on fat metabolism. Fred A. Abegg, Flemington, New Jersey. (Introduced by R. A. Brink.)
59. Diversities determined by intrinsic and extrinsic conditions in Stenostomum acaudatum (nov. spec.) with special reference to their inheritance. T. M. Sonneborn, National Research Council Fellow, Johns Hopkins University. (Introduced by H. S. Jennings.)
60. Flexed tail, a mutation in the house mouse. H. R. Hunt and Dorothy Permar, Michigan State College.
61. The effect of x-rays on the spermatozoa of Drosophila. L. G. Barth, University of Chicago. (Introduced by L. V. Heilbrunn.)

62. Blood relationship within the order Rodentia. R. A. Hicks, University of Michigan. (Introduced by C. C. Little.)
63. Malignancy and inbreeding. Leonell C. Strong, J. J. Bittner, Arthur M. Cloudman, University of Michigan.
64. The influence of environment on the inheritance of two characters in Drosophila. Mildred Hoge Richards, University of Oklahoma.

# ABSTRACTS

## 1. *An unexpected association of factors belonging to three linkage groups in Oenothera and its explanation.* George H. Shull, Princeton University.

In a family of Oenothera which was segregating for the three pairs of factors, rubricalyx vs. green hypanthia, long styles vs. brevistylis, and yellow vs. old-gold flowers, representing, respectively, the first, second, and third linkage groups, there was found a deficiency in the rubricalyx groups, due apparently to the linkage of the rubricalyx factor with a factor for semichloralbinism, together with the retarding effect on development, of the factor for old-gold flower color and short styles. There was, notwithstanding this partial elimination, a marked excess of the class showing the association of the three dominant traits, rubricalyx buds, long styles, and yellow flowers, representing a combination of three factors which had been associated in the maternal grandmother. This unexpected and striking excess of the traits of one grandmother seemed to imply the linkage of factors belonging to three different linkage groups—a condition which ought to be expected if the linkages in Oenothera are due to the end-to-end cohesion of non-homologous chromosomes. When preparing to test this suggested linkage, it was found that fifteen of the twenty-nine rubricalyx long-styled yellows were triploids produced by 14-chromosome eggs, which had failed to go through the reduction division, fertilized by 7-chromosome sperms. Omitting these triploids, the ratio of different phenotypes in the family no longer suggests a linkage between factors previously found to be independent.

## 2. *A case of doubling of chromosomes in the genus Nicotiana.* W. R. Singleton, Bussey Institution, Harvard University, and Connecticut Agricultural Experiment Station. (Introduced by E. M. East.)

A cross was made between Nicotiana rustica humilis Schrank and N. paniculata L., using the former as the female parent. The seeds from this cross germinate nearly 100 per cent, whereas the seeds from the reciprocal hybrid all fail to germinate.

The $F_1$ hybrid is intermediate, resembling more closely the rustica parent. It is only a fraction of a per cent fertile. Only a few of the flowers produce any seeds, and these never more than ten seeds per capsule.

About 200 $F_2$ plants were grown and examined for fertility and chromosome number. They ranged in fertility from 0 to 100 per cent. The chromosome numbers also proved interesting. N. paniculata has 12 (haploid), N. rustica has 24, and the $F_1$ has 12 pairs and 12 univalents, a total of 36 chromosomes. Several of the $F_2$ plants were apparently regular in both first and second reduction divisions and had 36 pairs, a total of 72 chromosomes—just twice the number in the $F_1$ plants.

These plants were in the most cases nearly completely fertile when selfed, or crossed by the rustica parent, but were completely sterile with the other parent, N. paniculata.

3. *New evidence bearing upon the problem of the cytological basis for genetical peculiarities in the oenotheras.* Ralph E. Cleland and Fr. Oehlkers, Goucher College.

The authors have made a study of the cytology and breeding behavior of a large number of $F_1$ hybrids between several species of Oenothera. They find that hybrids possessing circles composed of twelve or fourteen chromosomes breed true in practically every particular, whereas those with smaller circles and a number of paired chromosomes show a certain amount of splitting. It is thus shown that the chromosomes of a single circle constitute the basis for a single linkage group. It is further demonstrated that genes governing visible characters are scattered throughout the various chromosomes and are not confined for the most part to a single chromosome, as postulated by Shull.

4. *Inheritance of semisterility in maize.* R. A. Brink and C. R. Burnham, University of Wisconsin.

Semisterile maize plants appear identical with their normal sibs except that they produce 50 per cent aborted pollen grains and ovules. The matings normal ♀ × semisterile ♂, semisterile ♀ × normal ♂, and semisterile selfed give equal numbers of normal and semisterile offspring. It was suggested that in semisterile plants a section of one member of a chromosome pair is removed from its normal position and affixed to a non-homologous chromosome. The modified chromosomes apparently assort at random in meiosis, resulting in 25 per cent of the spores lacking the detached section and 25 per cent receiving it in duplicate. These classes abort. In one-half of the functional spores the section responsible for the atypical behavior of the stock is attached to a non-homologous chromosome. These spores transmit semisterility. According to hypothesis, the normal individuals in a given progeny from a normal × semisterile combination should give only normal offspring when crossed among themselves. Twenty-four such matings have given only normal offspring, with one exception probably due to contamination. The normal offspring from a selfed semisterile plant should comprise two classes of individuals in equal numbers, standard normals and x-normals. Crosses within each of these groups should give only normal offspring like the parents; intergroup combinations should produce all semisterile plants. Of ninety-one matings between normal sibs from selfed semisterile plants, forty-nine gave all normals and forty-two gave all semisteriles. The x-normal class is assumed to be 'homozygous' for the translocation. Semisterility is of particular interest as one of the mechanisms by which interspecific sterility may arise.

5. *A haploid mutant in the tomato.* E. W. Lindstrom, Iowa State College.

In an $F_2$ generation of 337 plants there occurred a single sterile tomato plant with an unusual morphological appearance. An examination of the pollen mother cells proved that only twelve univalent chromosomes were present, which was later verified by observations of somatic tissue of root tips. The genotype and phenotype of this exceptional plant showed it to be the result of the parthenogenetic development of a single, crossover gamete (factors in first chromosome). Thus far the haploid has proved completely sterile on the staminate side, but shows 1 to 3 per cent fertility when used as a female parent. The progeny from such crosses are diploid in nature, with one exception, and indicate that the functional megaspores are largely those containing the full quota of twelve chromosomes.

### 6. *The rôle of inhibiting factors in shape inheritance of squash fruits.* EDMUND W. SINNOTT, Barnard College, Columbia University.

Evidence is presented that in five lines of Cucurbita pepo, in which there is present one of the two major factors operating to flatten the shape of the fruit, there also occurs a dominant factor which inhibits the operation of either of the flatteners. This inhibitor is itself without direct effect upon shape and is not to be confused with factors which produce elongation directly.

### 7. *A lethal factor in Crepis effective only in an interspecific hybrid.* LILLIAN HOLLINGSHEAD, University of California.

Interspecific hybrids of Crepis capillaris and Crepis tectorum reported by Babcock and Collins ('20) developed only to the cotyledon stage. In renewed attempts to secure viable hybrids using different strains of the parental species one hybrid population was obtained, half of which were inviable, but half matured normally. Investigations showed that the tectorum parent had been heterozygous for a lethal which halted the development of hybrids into which it entered at the cotyledon stage. Selfed progeny of the tectorum plant showed no signs of the lethal, but on crossing the various plants to capillaris it was evident that the progeny were segregating for the lethal in a 1: 2: 1 ratio.

Plants homozygous for the lethal (ll) gave only inviable hybrids, those heterozygous for it (Ll) gave hybrids half viable, half inviable, and those homozygous for its allelomorph (LL) gave only viable hybrids. Progenies from plants of each genotype were grown and their constitution tested by crossing with capillaris. All the progeny of ll proved to be ll, giving inviable hybrids only. All the progeny of LL were LL, giving only viable hybrids. Progeny of Ll segregated in a 1 ll: 2Ll: 1 LL ratio giving hybrid populations of three kinds, 1) all inviable, 2) half viable, half inviable, 3) all viable.

The viability of the hybrids was independent of the capillaris parent and there was no differential germination with respect to the lethal.

There is evidence that the same lethal is effective in hybrids between tectorum and two other species, but hybrids with a third species are unaffected by it.

### 8. *The occurrence of chromosomal variants in Nicotiana alata.* T. H. GOODSPEED AND PRISCILLA AVERY, University of California.

Chromosome counts of some one hundred plants show that nine pairs is the characteristic number in Nicotiana alata. Differences in size, the position of constrictions, and the occurrence of satellites provide morphological distinctions between the nine pairs of somatic chromosomes. From a monosomic plant, obtained through controlled pollination, two populations, totaling seventy-nine plants, have been grown and examined cytologically. Of fifty-seven plants from normal ♀ × monosomic ♂, only two showed chromosome numbers other than 18, both plants having 19 somatic chromosomes. Of twenty-two plants from monosomic ♀ × normal ♂, five showed deviating chromosome numbers: 22, 25, 26, 27, 36. Of the seven plants with supernumerary chromosomes, only two, those with 22 and 25 chromosomes, showed marked distinctions from normal in external morphology. P. M. C. counts confirmed the chromosome number determined in root tips. Tetraploidy seen in the root tips of one plant was purely somatic, for

$9_{11}$ occurred at I-M. In one 19-chromosome plant, non-conjunction of one or two pairs of chromosomes frequently occurred, resulting in II-M counts of 8–11, instead of 9–10. This suggests that the high percentage of 8–10 II-M counts reported in N. alata may be due to non-conjunction as well as non-disjunction. At II-M considerable numbers of P. M. C. contained a single large spindle with 19 chromosomes, and diads constituted 12 per cent of the P. M. C. at the tetrad stage. It is suggested that non-conjunction, non-disjunction, and diploid gametes are responsible for the deviating chromosome numbers found.

*9. The frequency of somatic mutation in variegated pericarp in crosses of variegated with colorless maize.* R. A. EMERSON, Cornell University.

In $F_2$ of crosses of variegated with colorless maize, somatic mutations from variegated to self-colored pericarp occur more frequently in the heterozygous, VW, than in the homozygous, VV, ears. Different colorless races show differential effects on the mutability of the variegation gene. Colorless segregates from heterozygous light variegated plants increase the mutability of the variegation gene in crosses less than do the colorless races tested. Reciprocal crosses of variegated with colorless and of dark with light variegated stocks are not significantly different in mutation rate. In crosses of light with dark variegated stocks, $F_1$ is intermediate in variegation grade, neither parent type being dominant.

These observations are interpreted by the assumption of modifying genes linked with the variegation gene, which affect the mutability of the latter.

*10. Linkage between quantitative and qualitative genes in maize. I. Number of rows and cob color.* E. W. LINDSTROM, Iowa State College.

The entire lack of eight-rowed varieties of corn with red cobs led the writer to test for association between row number and cob color. A very large series of crosses of many varieties shows that, in $F_2$ and backcross generations, the distribution for row number on plants with red cobs is statistically different from those with white cobs. There is also a significant tendency for the eight-rowed segregates to possess white cob color. In view of other investigations with a similar situation this is interpreted to mean that one of the many genes controlling row number is located on the same chromosome (no. 6) with the general cob (and pericarp) gene.

*11. Some physicochemical aspects of life, mutation, and evolution.* JEROME ALEXANDER AND CALVIN E. BRIDGES, New York City and California Institute of Technology.

Many of the difficulties confronting those who have attempted to understand the nature of life and life processes arose from the fact that attention was not focused on sufficiently simple vital units. Even the smallest cell or monocellular being is an exceedingly complicated structure. In order to form a precise mechanical picture of what occurs in life, we must dissect down through the layered structure of its matter until we reach the simplest unit that can be regarded as living. The behavior of the tiny ultimate vital units may then be interpreted in terms of physicochemical concepts.

Starting with man as a unit generally conceded to be living, the microscope penetrates to the cell as a vital subunit. Cytology reveals as successively lower units, the nucleus and the chromosomes. Genetics demonstrates that the chromosomes are composed of hundreds or thousands of genes and suggests the possibility that the genes are compounded from gene elements or genels. The genes or genels are of the order of size and structure of small molecular groups or large molecules. The visible behavior of chromosomes in reproduction (mitosis), coupled with the genetic evidence, indicates that each gene forms a duplicate of itself next to itself, so that when the two gene strings split apart, under normal conditions each is an exact counterpart of the other.

The view is taken that genes are specific catalytic particles, which, in addition to having the power specifically to direct chemical change, have also the power to adsorb and fix selectively the subunits from which they are synthesized, and thus reproduce or duplicate themselves. This is accomplished by substantially the same method as ordinary catalysis, except that the catalyst is also the material whose formation is catalyzed.

If, prior to the self-duplication of a gene, the gene itself is modified by some non-lethal agent (x-rays, chemical substances), the gene or genes thus affected may reproduce steadily in the modified form, and the cell containing such modified material, as well as the clone or living being (biont) formed from it, will show consequences following on the change in nature and action of its controlling catalysts, the genes.

The existence of the so-called filter-passing or ultrafiltrable viruses and of bacteriophages indicates that there are whole series of subcellular bionts and points to the possibility that free-living particles may in some cases be of molecular rank. The first living thing was probably a molecule of this self-reproducing or autocatalytic type.

### *12. The rate of induced mutation in relation to dormancy, temperature, and dosage.* L. J. Stadler, University of Missouri.

About 90 per cent of the recognizable mutations induced by x-ray treatment of barley may be detected in the seedling stage. By using these as an index of mutation frequency, mutation rates for large populations may be determined. The conclusions here reported are based on mutation rates so determined in about 20,000 head progenies.

Mutation induced in dormant tissue: Mutation was induced by irradiation of dormant seed, but the mutation rate was much lower than that following similar treatment of germinating seed. Dormant seed are much more tolerant of irradiation than germinating seed, and under high dosage yielded mutations at a rate fully as high as that from germinating seed at their limiting dosage.

Mutation was induced in dormant seed whether or not the seeds were planted immediately after treatment.

Relation of mutation rate to temperature: No significant difference in rate of mutation was found in samples treated at 10°, 20°, 30°, 40°, and 50°C., in either dormant seed or germinating seed.

Relation of mutation rate to dosage: The rate of mutation, both in dormant seed and germinating seed, varies approximately in direct proportion to the intensity of irradiation. The average yield of mutations per Roentgen unit is about eight times as great in germinating seeds as in dormant seeds.

### 13. *Radium experiments with Datura. I. The identification and transmission of lethals of pollen-tube growth in $F_1$'s from radium-treated parents.* J. T. BUCHHOLZ AND A. F. BLAKESLEE, University of Texas and Department of Genetics, Carnegie Institution.

Radium-treated plants may carry lethal genes whose elimination takes place after pollination on the stigma or in the style. Many such plants were found and several selected for detailed study. An $F_1$ arising from treated pollen, normal appearing and having twelve bivalent chromosome pairs, was found to produce pollen in which more than half of the pollen grains remained ungerminated on the stigma. In some of the tests of this pollen a larger proportion of the pollen germinated, but was associated with a high proportion of burst pollen tubes in the style. Thus there was always an elimination of over half of the pollen.

The $F_2$ progeny from this plant appeared normal. When their pollen was tested, about three-fifths of twenty-eight offspring examined were found to be heterozygous for this gene. This is approximately a 1: 1 ratio and represents a condition inherited through the female gametophyte, though eliminated in the male gametophyte during pollen-tube growth, preventing the production of homozygotes in the $F_2$ generation.

Tests were made on nearly 200 $F_1$ plants from radium-treated parents. Many were found to be heterozygous for lethal genes affecting pollen germination or pollen-tube growth, and a number of other $F_2$ progenies were investigated. Controls: $F_1$ plants from treated stock which did not give tests for the presence of pollen-tube lethal genes gave rise to $F_2$ progenies which were likewise free from lethals. Pollen from untreated plants in the field in related strains were found to be normal.

### 14. *Radium experiments with Datura. II. Pollen abortion in $F_1$'s from radium-treated parents.* J. LINCOLN CARTLEDGE AND A. F. BLAKESLEE, University of Pittsburgh and Department of Genetics, Carnegie Institution of Washington.

From a total of 367 $F_1$ offspring from radium-treated daturas, the pollen of which was examined, two were new chromosomal types with, respectively, ± 50 and ± 75 per cent aborted pollen grains. Of the remainder which were normal in appearance, eleven showed ± 50 per cent abortion, one showed ± 85 per cent abortion, three had pollen in which about half of the grains were either distinctly smaller size or aborted, and one had half the grains lighter-colored from lack of starch. Thus, 4.9 per cent of the $F_1$ individuals showed at least half of the pollen grains abnormal. In addition, two plants had ± 25 per cent of the pollen aborted. Three normal-appearing plants with ± 50 per cent abnormal grains and one plant with ± 25 per cent abortion were tested by selfing. They all produced $F_2$ progenies which segregated for plants with normal and with abnormal pollen. As controls, 934 normal-appearing plants, the offspring of untreated parents, were tested. Only two of these showed as much as ± 25 per cent aborted pollen. Pollen abortion following radium treatment may be due to genes or to chromosomal abnormalities.

15. *Radium experiments with Datura. III. Chromosomal abnormalities in $F_1$'s from radium-treated parents.* A. DOROTHY BERGNER, SOPHIA SATINA, AND A. F. BLAKESLEE, Department of Genetics, Carnegie Institution of Washington.

Following radium treatment by Gager in 1921, nearly 18 per cent chromosomal mutants including the compound type Nubbin were identified. In line 1A, 0.47 per cent chromosomal mutants have appeared among over 15,000 individuals. Of fifty-two offspring grown in 1927 from radium-treated parents, two were new types, distinct morphologically, with deficiencies and other chromosomal abnormalities. Of the remainder which were normal in appearance, two had 10 bivalents plus a single ring of 4 chromosomes $(1 + M)$ and $(1 + m)$ and one a ring of 4 $(M + m)$ plus a chain of 4 $(L + M)$. This is a total of nearly 10 per cent with visible chromosomal abnormalities. In addition, from 333 similar plants in 1928, one out of seven examined had a ring of 6 chromosomes, and three plants not examined cytologically were morphologically distinct and probably abnormal chromosomally. The configurations of 4 attached chromosomes were identified in the $F_2$ in three cases. As controls, twenty-two offspring from an untreated parent showed only twelve bivalents. The attachment of chromosomes is attributed to induced interchange of parts between non-homologous chromosomes. Similar attachments have been induced in $F_1$'s between a standard line and various races from nature.

16. *Radium experiments with Datura. IV. Visible gene mutations in radium-treated and control material.* A. G. AVERY AND A. F. BLAKESLEE, Department of Genetics, Carnegie Institution of Washington.

Two recessive genes (swollen and yellow cotyledons) were obtained out of eighteen $F_1$'s tested from a capsule which had been treated with radium emanation by Gager in 1921. One recessive (pale leaves) was obtained out of fifty $F_1$'s from treatment of pollen or pollen tubes in 1927, making for the two experiments a total of 4.4 per cent of $F_1$ plants heterozygous for new genes. Two new recessive genes have been obtained following wounding (adherent and virescent). Haploidy seems to induce gene mutations, since out of 316 $F_1$'s from a haploid, 7, or 2.2 per cent, were heterozygous for new genes. As controls, from 568 line-1A plants in $F_2$ on later generations from a haploid, only one (0.18 per cent) was found heterozygous for a new gene. Except for this single plant, $F_1$'s from a haploid and the offspring of treated parents, no new genes have been discovered in normal plants in any of our cultures.

17. *Visible mutations from radium rays.* ELVENE WINKLEMAN, Washington University. (Introduced by Caswell Grave.)

18. *An analysis of the effects of the different rays of radium in producing lethal mutations in Drosophila.* FRANK BLAIR HANSON, Washington University.

Exposure of fruit flies to radium rays produces both visible and lethal mutations. Under heavy exposure, the lethals are so numerous as to lend themselves to exact quantitative work. A series of lead screens was interposed between the radium and flies. The thickest screen was 8 mm. thick and the thickness was halved each time until the thinnest screen was ⅛ mm. thick. The percentage of lethal mutations decreased with increasing thicknesses of lead, and the resulting curve was the same as that of the absorption power of lead screens for radium (Rutherford). Radium rays were then passed through the same series of lead screens into an ionization chamber and electroscope, and the amount of ionization measured. This is the physicist's method of measuring the amount of absorption taking place. The ionization curve and the mutation curve proved to be practically identical at all points, showing that the correspondence between the number of mutations and the amount of electrical energy is very exact.

*19. Chromosome translocations produced by x-rays in Drosophila.* H. J. MULLER, University of Texas, AND EDGAR ALTENBURG, Rice Institute.

Experiments were arranged to test the frequency with which translocations between non-homologous chromosomes are produced by x-rays in Drosophila. It was found that they arise with nearly the frequency of detectable gene mutations. They may involve attachments between any two or more of the chromosomes: X or Y, and II, III, and IV. The different breaks in a given chromosome may occur at different levels, as genetically determined. Cytological study of them has proved that, as previously suspected, different regions of the genetic map represent the chromosome to different scales, the map regions more crowded with genes representing relatively greater chromosome lengths; this does not, however, affect the validity of the linear order of the genes. When small pieces are translocated, individuals inheriting the loss without the translocation, or vice versa (thus having chromatin deficiency or excess), sometimes live, showing character changes that reflect the genic unbalance. Such cases have made it possible to prove that the left half of the X-chromosome is not sex-determining; probably, then, sex differentiation is accomplished chiefly by a single gene locus. Translocations (even when genically balanced) are commonly accompanied by lethal or deleterious character changes, like those of gene mutations, which make it difficult to obtain them homozygous. In one case a curious eversporting effect, involving more than one locus, arose in the translocated region, coincidentally with the translocation. Translocations can be produced with such regularity that several useful, prespecified translocation stocks, needed as tools in other Drosophila work, have already been constructed to order.

*20. Genetic effects of x-rays and other environmental agents.* ALEXANDER WEINSTEIN, University of Minnesota.

Among the mutations and translocations produced by x-rays are several of special genetic interest. One of the translocations involves at least three chromosomes—the X and the two large autosomes. Of the mutant genes, one, a second-chromosome dominant, causes a mottling of the eye, due apparently to mutation during development. The frequency of the mutation renders possible an analysis of how the process is affected by genetic influences (including sex) and by various environmental agents.

*21. The comparative effects of the same genes in different quantities.* ROBERT K. NABOURS, Kansas State Agricultural College.

Goldschmidt has recently reviewed the studies, mainly his own, of the effects of the same gene in different quantities. A clear case, not included in Goldschmidt's list, of the effects of single genes, and double quantities of them, respectively, among the color characteristics of Apotettix eurycephalus, is illustrated in Kansas Tech. Bull. 17, colored plate, matings 447, 455, et al., but otherwise not adequately elaborated. The factors, Y for a dominant large white spot on and T for a dominant mahogany red over the pronotum, have a linkage relation of about 7 per cent. Therefore, comparisons among the various combinations of these become easy. $\frac{Y \qquad +}{+ \qquad T}$, a single gene of each, shows the white spot of Y plainly modified by the mahogany of T; $\frac{Y \qquad T}{Y \qquad +}$, doubling the gene Y, with single T, exhibits the white spot conspicuously, with a mere tinge of the mahogany in it; $\frac{Y \qquad T}{+ \qquad T}$, doubling of T with single Y, shows the white spot greatly obscured by the mahogany. Many cases of this kind have been noted in the breeding of the grouse locusts.

22. *Wing production in aphids as affected by light and temperature.* A. FRANKLIN SHULL, University of Michigan.

If wingless females of Macrosiphum solanifolii are alternated daily between weak light and darkness, the curve of wing production in their offspring rises with increase of the duration of light to a maximum at about eight hours of light, falls slightly thence up to twelve hours of light, drops almost to zero at fourteen hours, and remains low for all longer daily periods. In stronger light the curve is higher for the short periods of light, but is nearly identical for the longer periods. Alternation between intense and weak light causes marked wing production only if the weak light is of very low intensity. Single exposures to light or darkness have small and irregular effects; repetition is necessary to obtain the above results.

Characteristic curves of wing production result from, 1) alternating between light and darkness in the time ratio of 1 to 2; 2) alternating twelve hours of darkness with various periods of light, and, 3) alternating six hours of light with various periods of darkness.

Temperatures must be not over 20°C. for at least part of the time to obtain these results. As the temperature rises above 20°, the effect of alternating light and darkness rapidly decreases until at 26°C. no wings are produced.

The explanation tentatively adopted is that some substance produced in the light is converted into another substance in darkness, and that this latter substance must be present in a certain amount to produce wings. Temperature modifies some part of the process.

23. *Radium experiments with Datura. V. Effects of radium on pollen-tube growth rate, and the seed output following treatment.* J. T. BUCHHOLZ AND A. F. BLAKESLEE, University of Texas and Department of Genetics, Carnegie Institution.

Pollen from normal $2n$ plants of Datura stramonium was subjected to treatment with radium-emanation tubes, using different dosages, and tested for rate of pollen-tube growth. There was found to be a decrease in growth rate consistently proportional to the dosage employed, the highest dosages resulting in a complete failure in the germination of the pollen. Data were obtained comparing the effects of a similar dosage in millicurie hours given by two tubes, one having four times the strength of the other.

Seed capsules resulting from pollinations with treated pollen were aborted without enlargement at high dosages and were aborted in stages after enlargement as the dosage was decreased. When still smaller dosages were employed, matured seeds were obtained and their number per capsule was increased with a decrease of dosage. Marked differences in seed output per capsule were obtained with dosage steps of 0.16 millicurie hour.

*24. A recessive factor for polydactylism in man.* Laurence H. Snyder, North Carolina State College.

Polydactylism, consisting of an extra fifth finger on each hand, was found among the negro population of Pamlico County, North Carolina. The three family histories given include 171 individuals, fifty-four of whom show the polydactylism. The character appears to be inherited as a recessive. It frequently skips generations. There are found eleven families in which polydactylous children occurred where both parents were normal. In one family, where both parents were polydactylous, all ten children had the character. The possibility of imperfect dominance has been considered, but does not seem to apply. There was no indication of sex linkage. Blood-group determinations were made on 120 of the individuals. These results were analyzed from the standpoint of linkage. The gene for polydactylism seems to be inherited independently of the blood groups.

*25. Types of supernumerary chromosomes in maize.* L. F. Randolph, Cornell University.

In maize have been found supernumerary chromosomes of three types: A) Duplicates of members of the typical set. B) Chromosomes of approximately the same size as the smallest members of the typical set, but differing from them in form and behavior. C) Diminutive chromosomes. Type-A chromosomes appear in the progeny of triploids crossed with diploids. Individuals possessing them are less vigorous than normal plants. In meiosis these chromosomes unite freely with the typical bivalents to form trivalent chromosomes. In root-tip cells they are indistinguishable from their homologues. Type-B chromosomes are present in many commercial varieties and genetical cultures and seem not to affect the visible characters of the plant: individuals with as many as ten or more of these chromosomes are vigorous and fertile. Chromosomes of this type rarely unite with the typical bivalents, but tend to form bi- and multivalent groups among themselves, and their meiotic distribution is extremely irregular. In root-tip cells they are club-shaped, thicker than the other chromosomes, and have a terminal fiber attachment. The appearance and behavior of these chromosomes indicate that they are homologous. Type-C chromosomes, discovered in black Mexican sweet corn, resemble those of type B in having uniformity of size, no marked effect on the plant, a similar meiotic behavior and manner of inheritance. They are larger than the satellites present in the typical set. Within the typical set are chromosomes with subterminal fiber attachments and with a long arm similar in length to the type-B chromosome, and a short arm similar to the type-C chromosome. A separation of the arms of such chromosomes may have produced these two types of supernumerary chromosomes.

### 26. *Studies on pigeon hybrids. II. The chromosome conditions as an index of differential mortality of embryos.* L. J. Cole, T. S. Painter, and Agnes Zeimet, University of Wisconsin and University of Texas. (Microscopic preparations.)

Hybrids were studied for the determination of their sex-chromosome constitution. Careful study of the germinal and somatic tissue of pigeons and doves (Streptopilia risoria) showed them very similar in their chromosome makeup, each species having among some sixty elements six large chromosomes in the male and five in the female. No other chromosomes approach these in size. Males have two pairs of V-shaped chromosomes, one pair being larger than the other, and one pair of rod-like elements. The sex chromosomes are the larger of the V-chromosomes. The female has only one of the larger V's and a pair each of the smaller V's and rod-like chromosomes.

Testes of nineteen adult hybrids have been studied. All these have the sex-chromosome constitution of males.

Cole's data show that some 60 per cent of the hybrids die after the first few days of incubation, such embryos undergoing certain well-marked changes prior to death. Out of a total of eleven embryos available and preserved to date, three showed characteristic signs of dying. Cytologically, these embryos possess the sex-chromosome constitution of females, i.e., they have only one of the large V-shaped sex chromosomes. The rest of the embryos are chromosomally males.

These results indicate, first, that the adult hybrids are genetically males and that none of them are transformed females, as has been claimed in some quarters. Secondly, that females are produced in the cross, but that they die in early embryonic stages.

### 27. *Chromosome morphology in nine- and ten-paired species of Nicotiana.* Priscilla Avery, Lillian Hollingshead, and Lillian J. Raup, University of California.

N. alata has 18 somatic chromosomes, distinguished by length, position of constrictions, and the occurrence of satellites: 2 pairs, long, with median constrictions; 2 pairs, medium, proximal satellites and subterminal constriction; 3 pairs, medium, with median or submedian constriction; 1 pair, medium, with subterminal constriction; 1 pair, shorter, with deep subterminal constriction. In plants with supernumerary chromosomes (19, 22, 25, 26, 27, 36), it is possible to recognize some of these chromosomal types duplicated. The somatic chromosomes of N. longiflora total 20 and show a characteristic arrangement at metaphase. Several chromosomes lie in a horizontal plane, arranged like the spokes of a wheel, the remaining chromosomes standing on end in the center of the plate. The chromosomes are rod-shaped, with only slight differences in length. Two pairs have constrictions forming distal heads. The fiber attachment on each chromosome appears to be truly terminal. These morphological distinctions in the somatic chromosomes of N. alata and N. longiflora are of interest because the sterile hybrid between these species forms $9_{II} + 1_{I}$ at I-M. N. Langsdorffii has 18 somatic chromosomes, which can be separated into four groups, as follows: 2 pairs, long, with median constriction; 1 pair, medium, with small proximal satellite; 1 pair with very large proximal satellites; 5 pairs showing slight differences in length. The large satellited chromosomes represent a striking morphological distinction between the somatic sets of N. alata and N. Langsdorffii. The $F_1$ between them is fertile and shows $9_{II}$ at I-M.

*28. Linkage of the characters albinism and shaker in the house mouse.* WILLIAM H. GATES, Louisiana State University.

In testing the new character shaker, reported by Lord and Gates (in press), for its linkage relationship to the other characters of the house mouse, a pink-eyed, short-eared, normal mouse was crossed with an albino shaker. The $F_2$ generation showed strong linkage with albinism. Out of an $F_2$ generation of 254, where expectancy is 144 colored non-shaker: 48 colored shaker: 48 albino non-shaker: 16 albino shaker, there were actually obtained 187 colored non-shakers: 3 colored shakers: 10 albino non-shakers: 54 albino shakers. The non-cross-over classes show an excess of 50 per cent and the cross-over classes a deficiency of 86 per cent. The differences are 15.5 and 15.9, respectively, times the probable error, and therefore quite significant.

The results of the back cross of the $F_1$ generation to the double recessive, albino shaker, are not complete, but up to date show a linkage of over 90 per cent.

This linkage relation gives the second group of three linked characters reported in the house mouse. The data, while extremely meager, seem to indicate that linkage is weaker between pink-eye and shaker than between albinism and shaker; and, therefore, it is likely that the chromosome map will show albinism lying between pink-eye and shaker.

*29. A comparison of the effect of extra chromosomes on pollen-tube growth from pollen of various ($2n + 1$) types of Datura stramonium.* J. T. BUCHHOLZ AND A. F. BLAKESLEE, University of Texas and Department of Genetics, Carnegie Institution.

A number of ($2n + 1$) types are known in which, *a*) the extra chromosomes may be transmitted through the pollen under favorable conditions. These usually have a bimodal distribution of the normal-appearing pollen tubes in the style, and genetic tests confirm the interpretation that the slower-growing group represents pollen tubes with ($n + 1$) chromosomes.

In other types, *b*) the pollen tubes are represented by two groups differing in appearance, a forward normal-appearing group and a group of abnormal-appearing tubes (swollen or ruptured). This condition is not transmitted through the pollen.

In other ($2n + 1$) types, *c*) half or slightly more than half of the pollen fails to germinate.

Pollen from Rolled, a ($2n + 1$) primary, gives the conditions described under *b*. One of its secondaries, Sugar-loaf, gives four classes of gametophytes, those which grow normally, a second normal-appearing, but more retarded group, a third group resembling in appearance and position the ($n + 1$) tubes of Rolled, and a fourth group represented by the 16 to 18 per cent aborted pollen. The first and second groups are the largest and occur in nearly equal proportions, while the third and fourth groups are smaller.

Polycarpic, the other secondary of Rolled, may have four groups, but only one (the first, with $n$ chromosomes) is capable of reaching the ovary. The Rolled group is recognizable by the appearance and position of the abnormal pollen tubes, while the pollen grains which might produce Polycarpic remain ungerminated on the stigma, and the aborted pollen constitutes a fourth group.

*30. Hybrid vigor in poultry.* D. C. WARREN, Kansas State Agricultural College.

For a comparison of the vigor of two pure breeds with that of hybrids resulting from their crossing, reciprocal matings were made between the single-comb White Leghorn and the single-comb Rhode Island Red breeds. Matings were so arranged that hybrid and pure-bred offspring were produced from the same females during each of the eight hatches. One hundred ninety-seven pure White Leghorns, 236 Rhode Island Reds, 173 hybrids from the cross Rhode Island Red male by White Leghorn female, and 176 from the reciprocal cross were produced. The percentage of mortality to the age of three weeks was 5.6 for White Leghorns, 6.3 for Rhode Island Reds, and 1.4 for the two reciprocal crosses combined. Hybrid males and females each outgrew the pure-bred offspring (of the same sex) from their mothers. Rhode Island Reds grew more rapidly than White Leghorns, and the cross of Leghorn male by Rhode Island Red female grew more rapidly than its reciprocal. The latter fact is of interest, since the slower-growing hybrids are from the cross in which the maternal parent was from the slower growing of the two breeds crossed. The Rhode Island Red breed reaches sexual maturity at a much greater age than White Leghorns. Females from the cross Leghorn male by Rhode Island Red female reached maturity at an age slightly greater than the White Leghorns, while the females of the reciprocal cross were more nearly intermediate with respect to age at maturity.

*31. Innate limitations of growth rate in the mouse.* E. C. MACDOWELL, W. H. GATES, AND C. G. MACDOWELL, Department of Genetics, Carnegie Institution of Washington, Cold Spring Harbor, Long Island, New York, and Louisiana State University.

The study of innate limitations of growth rate necessitates eliminating the effects of the secondary variables. To accomplish this, averages are usually relied upon, and much theorizing on the fundamental chemistry of growth has been based on averages of populations. But averages do not balance out the secondary variables, since these are of the nature of limitations and to the extent that any of these is working the nature of the innate processes is concealed. To remove the influence of these secondary limitations optimum conditions must be maintained.

As previously pointed out, prenatal life provides the most accurately controlled conditions for the study of growth in a mammal, and the growth curve of the mouse during this period forms a straight line on logarithmic paper when age is counted from the appearance of the anlage of the embryo. After birth, by increasing the amount of mothers' milk available and maintaining favorable conditions, we have been able to reduce the effect of the secondary limitations to such an extent that, for fourteen days after birth, curves for individual mice have been obtained that are nearly mathematically smooth, and rise to 15 grams, as against 4.5 to 7 grams in published data. These curves approach a straight line on logarithmic paper when age is counted from the anlage of the embryo. After fourteen days, a sudden break accompanied by a wide scatter among individuals indicates a change in the conditions of life. This is intimately associated with the first eating of solid food.

### 32. *Heredity of miniature (body), an x-ray mutation in Habrobracon.* P. W. WHITING, University of Pittsburgh.

Daughter of x-rayed male by untreated female gave rise to two mutations, short (wings) and miniature (body). Miniature is recessive and semilethal: females are sterile. About half of miniature males expected from heterozygous mothers fail to mature, but of these about 17 per cent develop into naked pupae, which can be classified with respect to eye color. Miniature shows linkage with orange (eye), giving about 10 per cent crossovers, as shown by mature normal, mature miniature, or pupae. There is some evidence that miniature is becoming more viable. Heredity of short is under investigation.

### 33. *Integumental grafting of the Leghorn fowl.* A. W. KOZELKA, University of Pittsburgh.

Secondary sexual structures from day-old chicks were transplanted onto various parts of body of same individual and of other individuals under three days of age. Autoplastic feather tissues differentiated normally on any part of body. Comb appeared to grow best in situ, but was usually somewhat smaller on other parts of body. While normal symmetry was maintained in most cases, shape of some of the grafted combs varied from irregular mass to a rosette. No innervation has taken place in grafted portions. Normal differentiation of autoplastic female spur took place only on scaly tissue of shank, remaining partially or wholly juvenile on other parts of body. In male, characteristic development of spur took place on any part of body, with one exception occurring among twenty-three grafts, where spur remained juvenile. Contrary to general belief, ovary appeared to have no inhibiting effect upon development of male spur. Of thirteen homoioplastic grafts with female as recipient, seven spurs from male donors differentiated into characteristic male spurs, while those from female behaved like autoplastic spurs. This would seem to indicate that there is genetic difference between spurs of two sexes. Work is now in progress to ascertain influence of sex hormones upon various secondary sexual characters.

### 34. *A third linkage group in the killifish, Platypoecilus.* ALLEN C. FRASER AND MYRON GORDON, Cornell University.

Previous reports have recorded a sex-linked group containing three factors: Sp, for black spots all over the body, R for red body color, and N, for the nigra pattern, a band of black (Bellamy, '22 and '28; Gordon, '27). The latter had also reported upon an autosomal factor St, for stippling, an olive gray coloration.

Since 1925, studies have been made on four other factors for certain melanic patterns occurring on the caudal peduncle of the fish: T, for twin-spot, Tb, for twin-blotch, O, for one-spot, and C, for crescent.

By a series of direct and reciprocal crosses and many back-crosses, the factors, T, Tb, O, and C indicate no linkage to St for stippling and are not sex-linked. They show in some cases definite linkage with one another.

Twin-spot by one-spot yields TtOo (or three spots). TtOo back-crossed to recessive (ttoo) gave 126 twin-spots, 109 one-spots, and a single cross-over TtOo. The latter is being tested further.

Evidence, although incomplete, indicates that C (crescent) and Tb (twin-blotch) belong to the same group as twin-spot and one-spot.

### 35. *A survey of flower colors in the garden balsam and of the genes affecting them.* Donald W. Davis and Lucy Ann Taylor, College of William and Mary.

Flower colors in the garden balsam occur in two series. Of the non-cream series our homozygous strains include the following colors associated with wholly green stems: white, nearly white (very slightly tinged with pink), nearly white with pink spur, pale pink with white spur, pink with pale spur (two types), pink with deep pink or purplish spur (two types), lavender (two types); and, associated with more or less pigmented stems, the following types: pink of various grades with deep colored spur (two types), scarlet (two types), purple, red, coppery red. In the cream series our homozygous stocks include pure cream, pale cream pink with cream spur, and cream lavender—all associated with green stems; and cream pink with deep spur (three types) associated with colored stems.

Sixteen crosses have been made involving ten of these twenty-five color types. Except in a few cases, these have been carried through the second, third, or fourth generation. Among the progeny of the crosses and from other sources eleven additional distinguishable color types are described.

White is a simple dominant to cream. Each of the other non-cream types, except the scarlets, red, and coppery red, has a recognizable corresponding cream type which differs from it by the same factor as differentiates white from cream. Scarlet is produced by a combination of the factors present in certain pale-spurred pinks with factors found in certain deep-spurred pinks. Purple results from a combination of factors found in lavender with those of certain deep-spurred pinks. Red is due to a combination of certain factors found in scarlet and purple. Details of crosses and their progeny give indications as to specific factors and their relations.

### 36. *A new type of congenital hypodactylism of the front limbs occurring in the sixteenth to nineteenth generations of the descendants of x-rayed mice.* Halsey J. Bagg, Cornell University Medical College and Memorial Hospital.

Four thousand three hundred sixteen animals were carefully examined for foot abnormalities before a sixteenth-generation individual was found with two digits of a front limb completely missing. In the next three generations (1279 additional mice examined) 37 individuals showed this general type of defect: males, 17; females, 20. There were 21 feet with one and 19 with two digits missing, respectively. The anatomical location was as follows: right front limb, 30 instances; left front limb, 4 instances, and three animals with both front limbs hypodactyl, respectively. Blindness was associated with this defect in 31 animals. Twenty-nine feet, excluding those that were hypodactyl, showed either clubbing or syndactylism. The solitary kidney condition was present in 7 animals and in 3 both kidneys were missing at birth. An examination of the breeding records shows that this type of hypodactylism new to the strain has arisen apparently in the grandchildren of two fourteenth-generation mice. These grandparents each had a clubbed foot, respectively, and the strain had been especially selected for this type of abnormality through the preceding six generations. It is possible that this is a distinct structural abnormality which arose possibly as a result of a new genetic recombination. The inheritance of this defect is being tested.

### 37. *On the several generations of daughters from repeated matings of daughters to sire.* H. D. GOODALE, Mount Hope Farm, Williamstown, Massachusetts.

The daughters of three White Leghorn males have been mated back to their sire, and the new generation of daughters in turn again mated to their sire. In one instance this has been repeated four times, the last generation of daughters theoretically averaging thirty-one thirty-seconds of the genes of their sire. Data relating to the body weight, fertility, and egg production of these daughters will be presented.

### 38. *Living mice and museum specimens showing morphological abnormalities of the viscera, eyes, and limbs.* HALSEY J. BAGG, Cornell University Medical College and Memorial Hospital.

Charts are shown illustrating the inheritance and association of the above defects, together with the results of a statistical study of a relative frequency in anatomical location, obtained from the examination of 5535 descendants of x-rayed mice through nineteen generations.

### 39. *Identity of genes and sex factors in intergeneric killifish hybrids.* ALLEN C. FRASER AND MYRON GORDON, Cornell University.

Kosswig ('28) states that in crosses of Xiphophorus helleri and Platypoecilus maculatus the sex-linked factors Sp, spots, and N, nigra, follow closely the normal hereditary behavior described for Platypoecilus, i.e., WZ type of sex inheritance; but sex-linked factor R was not consistent.

The gold Platypoecilus, one type Kosswig used, although having fins and back somewhat red, does not contain the dominant sex-linked factor R, for red body color (Fraser and Gordon, in press of Genetics). The secondary red, new factor Rf for red fins, when brought into combination with Xiphophorus factors, is intensified in $F_1$. Rf resembles R phenotypically, but is easily distinguished even when Rf is intensified. Rf has not as yet been shown in our experiments to be sex-linked.

In our back-crosses, two autosomal genes were tested: St, for stippling, and O, for one-spot on caudal peduncle, together with two sex-linked genes, R and Sp associated with the W chromosome. Platypoecilus ♀ ($W_{R\,Sp}\,Z_{r\ ps}$ Stst Oo) by $F_1$ hybrid ♂ ($Z_{r\ sp}\,Z_{r\ sp}$ Stst oo) gave: females, 11 red-spotted-stippled and 4 red-spotted; males, 4 stippled-one-spotted, 4 stippled, 1 one-spotted, and 1 recessive. Other back-crosses were made.

The back-crosses show stability of the $W_{R\,Sp}$ combination and simple mendelian inheritance of the autosomal factors.

### 40. *Multiple fission in Heteronema acus.* ROBERT C. RHODES, Emory University.

Typical binary fission in Heteronema acus is longitudinal. The reservoir system is halved, dividing from its posteriad region, near the anteriorly migrated nucleus. Doubt is held as to the method of perpetuating the two flagellae, but the older ones probably persist, one going to each daughter organism and two new ones originating by division of the basal granules. The old staborgan or mouth is, however, loosened, passes posteriad, and is resorbed, while new ones are differentiated from near the dividing reservoir opening.

In multiple fission, two, three, and sometimes four successive divisions of nuclear and cytoplasmic structures are accomplished without the complete separation of the cytoplasm of the daughter organisms. In some stages eight reservoirs are found with only six nuclei, two nuclei having probably been resorbed.

H. acus has been under continuous observation for eight years, and only once has multiple fission occurred, and this under unusual environmental conditions. We have not yet been able to induce it again. This occurrence may be abnormal and these somatellae largely monster forms; but whatever its explanation, it has not been previously recorded, so far as we know. The reduplication of reservoirs, flagellae, and mouth parts is significant, in that the daughter or newly originated cytoplasmic organelles are usually directly associated with nuclear division.

### *41. Heritability of the effects of ultraviolet and infrared waves upon the fission rate within the clone of Paramecium caudatum.* AUSTIN R. MIDDLETON, University of Louisville.

Of sixty Paramecium caudatum, all of the same generation from a single animal, twenty were exposed to ultraviolet light for fifteen days; twenty to infrared light for fifteen days, and twenty were cultivated as controls. The medium used was $\frac{1}{32}$ per cent Horlick's malted milk, prepared with distilled water. The fission rate was determined by balanced selection. The animals were 'washed' daily.

The three groups were continued for a second fifteen-day period under control conditions.

During exposure the ultraviolet group divided at the average rate of 8.15 fissions per line during fifteen days, with a range of from 3 to 12 fissions per line. During the following fifteen-day period they divided at the rate of 6.85 fissions per line, with a range of from 0 to 10 fissions.

During exposure the infrared group divided at the average rate of 30.15 fissions per line, with a range of from 28 to 32 fissions per line. During the following fifteen-day period they divided at the average rate of 18.6 fissions per line, with a range of from 11 to 16 fissions per line.

During the first fifteen-day period the control set divided at the average rate of 26.65 fissions per line, with a range of 23 to 31 fissions. During the second fifteen-day period they divided at the average rate of 13.3 fissions per line, with a range of from 10 to 20 fissions per line.

Therefore, in this clone, ultraviolet light apparently modified hereditarily the fission rate.

### *42. Four-strand crossing over.* ALEXANDER WEINSTEIN, University of Minnesota.

Crossing over has been shown to occur as originally described by Janssens: each chromosome is split lengthwise, but only two strands undergo interchange at any level. This necessitates a reexamination of the theory. The number of points of crossing over in the original strands of a tetrad and in the emerging strands do not necessarily correspond with each other or with the number in the tetrad as a whole. For example, a triple crossover tetrad might result in strands, none of which was more than a double crossover; and a triple crossover strand might result from a tetrad in which no original strand had crossed over at more than two points. The true frequency of crossing over for either strands or tetrads would on certain assumptions be—

$$X = \left(\frac{1}{p}\right)^r \left[a_0 - \left(\frac{1-p}{p}\right)a_1 + \left(\frac{1-p}{p}\right)^2 a_2 - \left(\frac{1-p}{p}\right)^3 a_3 + \ldots\ldots + \left(-\frac{1-p}{p}\right)^n a_n\right].$$

This formula would, in general, give values of crossing over different from the ordinary ones; but the theory of linear arrangement would remain valid. One type of coincidence would on certain assumptions remain invariant; but other types would not. The application of the formula to experimental data (including ordinary cases and cases involving non-disjunction, attached X's, and polyploids) makes possible a test of the assumption on which it is based and shows that the association of strands in crossing over is not a matter of pure chance.

*43. Eye defects in rabbits.* HEMAN L. IBSEN AND L. D. BUSHNELL, Kansas State Agricultural College.

In 1924 we began our attempts to duplicate the work of Guyer by treating rabbit females (and males) for the purpose of affecting the eyes of their offspring. Ten females were injected with rabbit anti-lens serum obtained from hens; they produced fifty-eight normal-eyed offspring and one with a slight cloudiness at the base of a lens (observed with an ophthalmoscope). In the remaining experiments one or both eyes of the females, and in certain cases both eyes of the males, were needled. When only the females' eyes were needled, there were 130 offspring produced, all with normal eyes. When both parents had their eyes needled, fifty-five of the offspring were normal-eyed and one had a small spot on the surface of each cornea near the center of the pupil (visible to the naked eye). Many of the normal-eyed offspring from treated parents were mated. The records on their 158 descendants show that all of them were normal-eyed.

At about the time the above experiments were begun, two animals with noticeably defective eyes were born in the laboratory from untreated stock. During the progress of the experiments three more of similar parentage were born. One of the latter was produced when a defective-eyed male was mated to his normal-eyed sister. All of the five above mentioned were much more noticeably defective than the two obtained from treated stock.

*44. Twinning in some fresh-water snails.* EDWARD D. CRABB, University of Pennsylvania.

The object of this investigation was to gain information on the question of mon-ovular or bi-ovular origin of twins. The maximum number of vitelli, or embryos, found in a single egg was two in the Jap snail (Viviparus malleatus), Physa sayii, and P. ancillaria; five in the Potomac snail (V. contectoides); eight in Lymnaea palustris, and forty-six in L. stagnalis appressa. Over 200 polyvitelline Lymnaea eggs were incubated to advanced stages or used in breeding experiments without obtaining a single mirrored-image, or sinistral, individual or any indication that laying these eggs is a heritable character. In L. s. appressa the percentage of polyvitelline to normal eggs was 273: 19,863; in L. palustris, 15: 6939 (in another instance, 27: 65 cc. of 'dry' egg masses), and in Physa sayii, 2: 9061. The abnormal eggs were studied entire and in sections. There are two instances in which two fused mature vitelli, in stages before the first cleavage, were found. The presence of polar bodies on both fused vitelli shows that each of these potential conjoined twins arose from two ova. Studies of whole and sectioned eggs show that maturation in each vitellus was normal and that cleavage was quite as independent as in normal eggs. There is no evidence for mon-ovular origin of the twins studied.

*45. The determination of identity in twins.* S. J. HOLMES, University of California.

The determination of identity in twins cannot be made with much probability from any one character or even by an enumeration of points of resemblance unless these are of an exceptional kind. In order to secure a more reliable index of identity, the measurements of particular parts in each pair of twins were reduced to a quotient by dividing the smaller by the larger measurement. The nearer this quotient approaches unity the closer the resemblance of the twins in the trait in question. Quotients were calculated for thirteen characteristics that were measured. Then all of these quotients were multiplied together to secure a general index of resemblance. This procedure gives weight to all factors involved, and at the same time it tends to compensate, to a certain extent, for any unusual difference in the measurements of any one characteristic.

Applying the test of this composite coefficient of resemblance to a group of twins makes it apparent that the several pairs tend to fall into two fairly contrasted categories. In early years there is little overlapping of the two groups, but the differences become less sharp as the twins increase in age. By the use of this coefficient one may ascertain from the extent of divergence accompanying age something of the relative potency of environmental factors in the production of the similarities and dissimilarities between human beings.

*46. Sex determination and sex-ratio determination in Sciara.* CHARLES W. METZ, Department of Genetics, Carnegie Institution of Washington.

In some species of Sciara, pair matings regularly give 'unisexual' progenies and in other species 'bisexual' progenies. Genetic experiments on former type show that female is responsible for the sex ratio. Female-producing female regularly gives female-producing and male-producing daughters in equal numbers, suggesting the hypothesis that genetic constitutions are as follows: female-producing female, Aa; male-producing female, aa; male, aa. This is supported by the fact that 'exceptional' females from male progenies are male producers (twenty-two tested.)

Genetic evidence from sex-linked mutant character in S. coprophila indicates presence of sex-chromosome pair—XX female XY male. Hence sex is presumably 'determined' by sperms, of which each male produces both kinds. Since females give 'unisexual' progenies and since preliminary egg counts indicate absence of selective mortality, evidence suggests presence of selective fertilization —eggs of female-producing females being susceptible to fertilization by X bearing sperms only, and vice versa.

As previously noted, cytological study of several species of Sciara shows that males typically have two more chromosomes than females—the large 'sex-limited' chromosomes. These apparently pass into all sperms. In production of females they are presumably eliminated at or after fertilization. That they are not primary agents in sex determination is indicated by genetic data mentioned above and by the fact that in cytological material of one species recently studied they are lacking.

### 47. *The establishment of races of pigeons characterized by large or small thyroids.* Oscar Riddle, Carnegie Institution of Washington.

It was previously reported that at least two 'small thyroid' and two 'large thyroid' races had been established. Those results have now been extended to include four races of each type. Through four to six generations the healthy individuals of these races or strains have satisfactorily maintained their thyroid size at a characteristic level. It is thought that the numerous difficulties attending a study of the heritability of thyroid size have been adequately eliminated in our study of twenty-four strains of ring doves.

The fact that 'thyroid races' have been formed is in itself evidence that genetic factors for thyroid size do exist. Additional evidence is now available from crosses of the various races. Large thyroid × large thyroid yields large thyroids in $F_1$. Small thyroid × small thyroid gives small thyroids in $F_1$. Large thyroid × small or intermediate thyroid gives thyroids of intermediate size in $F_1$. Some further evidence is obtained from smaller numbers of $F_2$ and $F_3$. Questions of dominance and of the number of genetic factors involved require further study.

### 48. *Studies on pigeon hybrids. I. Aberrant sex ratios.* L. J. Cole, T. S. Painter, and Agnes Zeimet, University of Wisconsin and University of Texas.

Males are commonly in excess among hybrid animals. Whitman and Riddle demonstrated this for pigeon hybrids, the proportion of males being in general higher the wider the cross. Two principal explanations have been offered: 1) sex reversal and, 2) differential death rate of embryos. An attempt has been made to determine which is correct by studies of, 1) the death rate of embryos; 2) sex-linked characters, and, 3) chromosomal relations. The present report deals mainly with the death rate.

Domestic pigeon (Columba livia) ♂ × Barbary dove (Streptopilia risoria) ♀: Of 1053 eggs, 575 are reported as fertile. It was soon apparent that there was a very large death of embryos within the first few days of development. Of those continuing development, 222 are recorded as males and three as females. It is evident that because of the high early death rate the possibility that the aberrant sex ratio may be due to differential mortality at that time is not excluded.

Similar results were obtained from the cross of Chinese Pearlneck dove (Spilopilia chinensis) ♂ with Barbary dove ♀, but in this case the relationship is closer and the excess of males produced is not so high.

In so far as it has been possible to check up on sex-linked characters, the results are in accord with the interpretation that the sex of the adult is what would be expected on the genetic basis. In other words, no evidence of sex reversal has been found.

### 49. *The critical period for sex control and the possible mechanism of sex determination in Cladocera.* Arthur M. Banta, Department of Genetics, Carnegie Institution of Washington, and L. A. Brown, Department of Zoölogy, University of Iowa.

Mothers crowded or otherwise subjected to treatments which markedly lower their rate of development produce a large percentage of males among their parthenogenetic offspring, whereas control mothers under normally favorable conditions rarely produce any males. By beginning the crowding of the mothers at various times before egg laying and, on the other hand, by stopping the crowding at similar intervals before egg laying, the critical period for control of sex was localized as a brief interval at approximately four hours (at 20°C.) before the eggs are laid. Previous to this period the sex of the forthcoming young is subject to control measures; after this period sex is fixed. The critical period is about three and one-half hours before the spindle for the single maturation division appears.

Control of sex in Cladocera is assumed to operate through a sex-determining mechanism, although such a mechanism is cytologically unknown in this group. The possible type of sex-chromosome mechanism involved is discussed.

### 50. *The functional relation of the genes in the X-chromosome of Drosophila melanogaster, referred to bar eye as base.* A. H. HERSH, Western Reserve University.

By the use of the stock Xple (scute, echinus, crossveinless, cut, vermilion, garnet, forked) and Zeleny's low selected forked-bar stock, different combinations of the genes were made by suitable matings. The mutant genes of the Xple stock were added singly to forked bar, and cumulatively in the left-right direction and also in the right-left direction on the chromosome. The facet counts of these fifteen stocks, raised at 25°C., show that when the genes are added singly cut and garnet are fairly strong minus modifiers of facet number, the others are plus modifiers, crossveinless and vermilion notably so. When the mutant genes are added cumulatively in the left-right direction on the chromosome, bar eye becomes progressively larger until garnet is added. With the addition of garnet, that is in Xple bar, the eye is but slightly larger than in forked bar. When the sections of the Xple chromosome are added to forked bar in the right-left direction on the chromosome, the eye is smaller than in forked bar, but becomes progressively larger, never, however, reaching the size of forked bar until all are added. The logarithm of the number of facets in the dorsal lobe (x) is directly proportional to the logarithm of the number in the ventral lobe (y) of the eye, that is, $y = bx^k$. A comparison of the values of $b$ and $k$ for the fifteen different combinations, calculated by a least-squares method, indicates that $b$ shows an exponential decrease of about 44 per cent for each 0.1 increase in $k$.

### 51. *The inheritance of egg weight in domestic fowl.* F. A. HAYS, Massachusetts Agricultural College.

Studies are based on egg-weight records made on Rhode Island Red hens hatched from 1924 to 1927. The total number of birds upon which records are available is 593.

The data indicate a very rapid increase in the weight of eggs laid by pullets between the first egg laid and the egg weight of the same individuals by the first of March. It has been shown to be possible to predict the probable egg weight of birds during the spring hatching season by weighing the eggs in November or December previous. Maximum egg weight for the flock studied appeared during the second laying year.

The market system of classifying eggs as 'standard' if they weigh 56.7 grams is essentially sound biologically, and birds laying such eggs differ genetically from those laying small eggs.

A dominant autosomal gene B for large eggs and a similar gene A for small eggs appear to be concerned. Gene A is hypostatic to B. Both show cumulative effects and no sex linkage is observed. Recessive birds lay very small eggs, not differing greatly in size from those of Gallus bankiva.

*52. The effect of long subjection to constant temperatures upon bar eye of Drosophila melanogaster.* CHARLES ZELENY, University of Illinois.

Under a constant temperature of 27°C. low selected and inbred bar of Drosophila melanogaster has undergone a significant increase in eye-facet number during the course of 134 generations in sixty-seven months. During the same time in fifty-one generations at 17° the same stock has not changed in this respect. Reciprocal transfers between the two temperature lines demonstrate, however, that the temperature coefficients have decreased the same amount in each.

*53. The temperature coefficient and temperature-effective period for wing size in Drosophila.* WILLARD F. STANLEY, University of Illinois. (Introduced by Charles Zeleny.)

The length of vestigial wing varies directly with the temperature, but not in direct proportion. The length of the normal, full wing varies inversely with the temperature. There is a distinct sexual dimorphism of wing size in vestigial wing which is not consistently in one direction at all temperatures, but is dependent upon the specific temperatures. There is also a distinct sexual dimorphism in per cent increase or decrease in wing length and in the sex temperature coefficients. This marked sexual dimorphism suggests the effect of the various temperatures upon possible accessory factors on the X-chromosomes. The temperature-effective period for vestigial wing has been positively demonstrated. There appears to be a distinct sexual dimorphism as regards the limits of the effective period. The data indicate that the effective periods at 17° and 27° are approximately in proportion to the total developmental periods at those temperatures.

*54. The effect of temperature on infrabar and its heterozygotes.* W. M. LUCE, University of Illinois. (Introduced by Charles Zeleny.)

In the bar series of multiple allelomorphs of Drosophila melanogaster the number of facets in the compound eye is dependent upon the temperature at which the larvae are grown. By means of facet counts of flies transferred at stated intervals from one temperature to another, it was demonstrated in homozygous infrabar and in infrabar by bar heterozygotes that the effective period is confined to a comparatively short part of the life-cycle, corresponding roughly to the latter half of the larval period. At a corresponding time in development the imaginal discs of the adult eye are being formed so that it seems probable that the effective period for temperature is confined to the period of rapid growth and multiplication of cells in the imaginal discs before the onset of differentiation into the final form.

In the heterozygote the effect of temperature upon the acceleration of the velocity of the facet-forming process is very nearly the same as the acceleration in general larval development produced by temperature. The major effect of temperature on facet number is in this case upon the processes that initiate and terminate the effective period. On the other hand, in infrabar the major effect of temperature upon facet number is the great acceleration in the facet-forming process caused by this agent, while the differential effect upon the processes that initiate and terminate the effective period is slight.

*55. The occurrence of parthenogenesis in the genus Chrysanthemum.* EDWARD C. JEFFREY, Harvard University.

The genus Chrysanthemum has long been interesting on account both of the extreme variability of some of its species and also by reason of the polyploidy which it likewise exemplifies. Experimental and morphological studies of the genus show that many of its species are parthenogenetic in their mode of reproduction. This mode of reproduction is quite commonly associated in plants with polyploidy, as, for example, the Canina section of roses. C. leucanthemum is tetraploid and parthenogenetic and appears to typify other species of the genus in respect to the association of polyploidy with parthenogenesis.

*56. The inheritance of reproductive characteristics in Daphnia longispina.* KATHRYN ORDWAY, Department of Genetics, Carnegie Institution of Washington. (Introduced by Arthur M. Banta.)

Inasmuch as parthenogenesis in Cladocera occurs without chromatic reduction, all the members of a clone should have the same inheritance. On the other hand, sexually produced young arising from a cross between two clones show hereditary differences in morphological characteristics. In this study, material from Doctor Banta's laboratory was utilized to obtain further data concerning the inheritance of reproductive characteristics in Daphnia longispina. Series of mothers from sister clones (originating from hatched individuals from a cross between two clones) were simultaneously observed concerning their reproductive characteristics as indicated by: 1) age at time of beginning reproduction; 2) average number of young per brood; 3) average number of broods per mother; 4) average interval between broods; 5) reproductive index (average number of young per mother per day), etc. Several repetitions of such series were made. In all, in the three different groups of related clones studied, a total of 565 mothers and 78,825 young were observed. As judged by the averages obtained from the various measures employed, reproductive characteristics are markedly uniform for the different members of a single clone (whether studied in only one or in different parthenogenetic generations). On the other hand, sister clones differ widely from one another; the same intraclonal constancy is apparent, but interclonal differences are of wide range and are of like order and magnitude in different series of mothers cultured and studied simultaneously in several parthenogenetic generations. The data obtained indicate that reproductive characteristics (and longevity) in Daphnia longispina are determined by genetic factors and that several such factors are involved.

*57. Inheritance in Mongolian idiocy.* MADGE THURLOW MACKLIN, University of Western Ontario Medical School.

A critical review of 1408 cases of Mongolian idiocy published in the literature lends no support to the theories that environmental influences play an aetiological rôle in its production. Thus syphilis, mental suffering of the mother during pregnancy, advanced age of either parent, especially the mother, at the time of conception, great difference between the ages of the parent or the number of preceding pregnancies—none of these has any part in causing this disease.

Heredity is the causative factor—a statement borne out by the study of the occurrence of Mongolian idiocy in twins. It is not dependent upon the presence of a pair of unit recessive factors, as has been stated, but is dependent upon a complex grouping of factors, presumably recessive.

*58. Some effects of the waxy gene in maize on fat metabolism.* **Fred A. Abegg**, Flemington, New Jersey. (Introduced by R. A. Brink.)

A more complete determination of the specific effects of the waxy gene on reserves in maize grain and pollen would seem to be a prerequisite in establishing the basic physiological activities of this heritable element during development. The evidence as cited indicates that the waxy gene modifies the fat metabolism of maize endosperm and that this gene is not limited in its sphere of action to changes in carbohydrate metabolism. The crude fat yield of non-waxy endosperm tissue proved to be 1.05 per cent; that of related waxy endosperm tissue, 1.5 per cent. The corresponding embryo tissues gave crude oil yields of 34.0 and 34.2 per cent, respectively. The average acid value of the crude fat from non-waxy endosperm tissue was found to be 45 and that of closely related waxy, 82. In the same stock of material the average acid value of non-waxy embryo crude oil was 4.21 and that of waxy, 4.16. The average saponification value of non-waxy endosperm crude fat was found to be 177 and that of waxy, 200. Related ester values were 117 and 100, respectively. Non-waxy and waxy pollen are nearly equal in crude fat content and differ little in acid values. Marked differences between these types are characteristic of ether extracts from endosperm tissues only. In some manner the waxy gene apparently reduces the synthetic power of waxy endosperm lipase, hence the acid value of the waxy endosperm crude fat is higher than that of the non-waxy.

*59. Diversities determined by intrinsic and extrinsic conditions in Stenostomum acaudatum (nov. spec.), with special reference to their inheritance.* **T. M. Sonneborn**, National Research Council Fellow, Johns Hopkins University. (Introduced by H. S. Jennings.)

This rhabdocoel, reproducing asexually, reveals intrinsic individual differences determined by the position in the chain of zooids. A series of successive anterior products of reproduction exhibits a course of structural and physiological changes terminating in death; a series of successive posteriors undergoes neither such changes nor death. The series of successive anteriors may be considered a single parent which ages and dies after budding off numerous zooids from its posterior end. Parents differ from their offspring by budding more slowly (compared on the same dates) and by dying sooner.

Differences (of extrinsic origin) due to the action of dilute (0.0025 per cent) lead acetate on single individuals have been undemonstrable. Results of action of the lead acetate on a succession of generations are: 1) an increase in the time to produce succeeding generations; 2) the production of structural abnormalities. The action is cumulative (that is, inherited) from generation to generation during treatment.

After removal to normal conditions, structurally abnormal individuals produced in this way and others arising from aging parents under normal conditions have the following fates: most, 1) die or, 2) become normal; a few, 3) retain their abnormality permanently. The progeny of abnormals are: generally, 1) normal; sometimes, 2) recipients of the parental abnormality by pseudo-inheritance;

rarely (not at all, among the progeny of aging normals), 3) identically like the parents, forming a new biotype.

*60. Flexed tail, a mutation in the house mouse.* HARRISON R. HUNT AND DOROTHY PERMAR, Michigan State College.

Two males showing this mutation were found in an albino stock secured originally from Dr. W. E. Castle. Eighteen flexed-tailed descendants of these two males were mated with numerous normal-tailed mice. All of the 316 $F_1$'s were normal, showing that the mutation is recessive. The $F_2$'s comprised 423 normal males, 487 normal females, 49 flexed-tailed males, and 83 flexed-tailed females—a total of 1042. The $F_2$ ratio is 6.9 normals: 1.0 flexed-tailed. Eight crosses of flexed × flexed have produced 60 young, all of them flexed-tailed. Back crosses are being made between $F_1$ females and their flexed-tailed male parents. An experimentally confirmed explanation is still being sought for the peculiar $F_2$ ratio.

The character varies considerably. In all cases the tail is permanently rigid over a varying portion of its length, this stiffness being particularly conspicuous proximally. The rigidity may be accompanied by permanent V-shaped, U-shaped, spiral, etc., flexures, though sometimes the character may be identified only by the inflexibility of a limited section of the tail. Further studies of the mutation are in progress.

*61. The effect of x-rays on the spermatozoa of Drosophila.* L. G. BARTH, University of Chicago. (Introduced by L. V. Heilbrunn.)

X-ray treatment of males of Drosophila produces a deviation in the sex ratio of their progeny in the direction of more sons when the males are mated with normal females. This deviation from a 1: 1 ratio amounts to 6.7 times the probable error. When the x-rayed males are mated with females having attached X-chromosomes, a deviation from a 1: 1 sex ratio of 13.4 times the probable error is obtained. In this mating more daughters are produced than sons. The two results are consistent and serve as a check on each other, for in the first case the sons are determined by the union of a Y sperm and an X egg, while in the latter mating the daughters are determined by the Y sperm and an XX egg. Thus in both cases there are more individuals resulting from fertilization by Y sperm than X sperm. Therefore, there exists a differential susceptibility of the X and Y sperm to x-rays, resulting in a deficit of functional X sperm.

*62. Blood relationship within the order Rodentia.* R. A. HICKS, University of Michigan. (Introduced by C. C. Little.)

In his classic study of phylogeny by means of precipitating antisera, Nuttal left untouched the problem of the blood relationship of the rodents. The present serological study of the four species of mice, Mus musculus, Mus wagneri, Mus faeroensis, and the Japanese waltzing mouse of the genus Mus was begun with an attempt to produce precipitating antisera in pigeons, using the serum or muscle extracts of these rodents as antigens. In spite of all modifications in manner of treatment of the birds, the study of 200 pigeons has not given an antiserum which would differentiate these mice. At present the pigeon may be considered unsuited for the production of precipitating antisera.

Recently, acting upon the suggestion of Dr. F. G. Novy and following the work of Uhlenhuth and Weidanz ('09) and R. Trommsdorf ('09) rabbits have been immunized against the serum of Mus musculus. Precipitating antisera have been obtained from these rabbits and have been found to be specific within the order Rodentia.

The rabbit has been demonstrated to be free from natural mouse or rat precipitin and is suitable for the production of these antibodies. The four species of mice studied fall into two groups serologically distinct, one being Mus musculus and Mus faeroensis and the other Japanese waltzer and Mus wagneri. Inside either of the two groups it has not been found possible to make any serological distinction.

### 63. *Malignancy and inbreeding.* Leonell C. Strong, J. J. Bittner, and Arthur M. Cloudman, University of Michigan.

Several years ago many individuals of a moderately inbred and relatively homogeneous stock of mice (Bagg albino) gave rise to spontaneous tumors. The diagnoses, although the tumors varied slightly in histological appearance, were mostly carcinoma. These tumors failed to grow in any other individuals (a large number of Bagg albino mice were used for inoculation). In comparison, spontaneous tumors arising in the highly inbred dilute brown stock will grow in 100 per cent of the dBr strain, even at the initial inoculation, although the degree of malignancy of dBr tumors is estimated histologically to be lower than that of the albino tumors. It is therefore hard to see what genetic relationship really exists between malignancy and transplantability. Since the first results, the process of inbreeding Bagg albinos has been continued. Recently, upon testing out transplants of new spontaneous tumors arising in that strain, we find that they will grow in 100 per cent of inoculated individuals, even at the initial inoculation. Thus it would seem that genetic factors, segregated during the process of inbreeding, undoubtedly play a very important rôle not only in the assumption of malignancy by, but also in the transplantability of, the tumor cell.

### 64. *The influence of environment on the inheritance of two characters in Drosophila.* Mildred Hoge Richards, University of Oklahoma.

Eggs of ten mutant races of Drosophila melanogaster were subjected to temperatures of 10° and 14°C. From the eggs so treated 5.5 per cent of abnormal flies hatched, but the new characters did not persist to any extent in subsequent generations. Further experimentation with the descendants of these treated stocks brought out frequently two characteristics. Selection of these two, abnormal abdomen and blistered wings, developed relatively small percentages of the abnormalities. An attempt was made to determine the influence of the environment on these two characters. Treatment with cold temperatures brought out a much larger per cent of blistered wings, but failed to affect abnormal abdomen. When the eggs were developed under moderately moist conditions, the abnormal abdomen stock produced a much greater number of flies with the abnormality. Abnormal abdomen is not sex-linked; blistered wings is apparently sex-linked and semilethal.

# AMERICAN SOCIETY OF ZOÖLOGISTS

## CONSTITUTION, OFFICERS, AND MEETING PLACES OF THE SOCIETY

## CONSTITUTION

### Article I

### NAME AND OBJECT

*Section 1.* The Society shall be called the "American Society of Zoölogists."

*Sec. 2.* The object of the Society shall be the association of workers in the field of Zoölogy for the presentation and discussion of new or important facts and problems in that science and for the adoption of such measures as shall tend to the advancement of zoölogical investigation in this country.

### Article II

### MEMBERSHIP

*Section 1.* Members of the Society shall be elected from persons who are active workers in the field of Zoölogy and who have contributed to the advancement of that science.

Zoölogists without the training in research required for full membership may be elected associate members of the Society.

*Sec. 2.* Election to membership in the Society shall be upon recommendation of the Executive Committee.

*Sec. 3.* Each member shall pay to the Treasurer an annual assessment as determined by the Society. This assessment shall be considered due at the annual meeting and the name of any member two years in arrears for annual assessments shall be erased from the list of members of the Society, and no such person shall be restored to membership unless his arrearages shall have been paid or he shall have been re-elected.

*Sec. 4.* Foreign Zoölogists, not members of this Society, may be elected Honorary Fellows upon unanimous recommendation of the Executive Committee by a majority vote of the members present at any meeting of the Society. Honorary Fellows shall not be required to pay dues.

### Article III

### OFFICERS

*Section 1.* The officers of the Society shall be a President, a Vice-President, a Secretary and a Treasurer and the members at large of the Executive Committee.

*Sec. 2.* The Executive Committee shall consist of the President, the Vice-President, the Secretary, the Treasurer and five members elected from the Society at large. Of these five members, one shall be elected each year to serve five years. If any member at large shall be elected to any other office, a member at large shall be elected at once to serve out the remainder of his term.

*Sec. 3.* These officers shall be elected by ballot at the annual meeting of the Society and their official terms shall commence with the close of the annual meeting, except that the Secretary and the Treasurer shall be elected triennially and shall serve for three years.

*Sec. 4.* The officers named in Section 1 shall discharge the duties usually assigned to their respective offices.

*Sec. 5.* Vacancies in the board of officers, occurring from any cause, may be filled by election by ballot at any meeting of the Society. A vacancy in either the Secretaryship or the Treasurership occurring in the interval of the meetings of the Society may be filled by appointment, until the next annual meeting, by the Executive Committee.

*Sec. 6.* At the annual meeting the President shall name a nominating committee of three members. This committee shall make its nominations to the Secretary not less than one month before the next annual meeting. It shall be the duty of the Secretary to mail the list of nominations to all members of the Society at least two weeks before the annual meeting. Additional nominations for any office may be made in writing to the Secretary by any five members at any time previous to balloting.

## ARTICLE IV

### MEETINGS OF THE SOCIETY

*Section 1.* Unless previously determined by the Society the time and place of the annual meeting of the Society shall be determined by its Executive Committee. Special meetings may be called and arranged for by the Executive Committee. Notices of such meetings shall be mailed to all members of the Society at least two weeks before the date set for the meeting.

*Sec. 2.* Sections of the Society may be organized in any locality by not less than ten members, for the purpose of holding meetings for the presentation of scientific papers. Such sections shall have the right to elect their own officers and also associate members; provided, however, that associate membership in any section shall not confer membership in the Society.

## ARTICLE V

### QUORUM

Twenty-five members shall constitute a quorum of the Society and four a quorum of its Executive Committee.

## ARTICLE VI

### CHANGES IN THE CONSTITUTION

Amendments to this Constitution may be adopted at any meeting of the Society by a two-thirds vote of the members present, upon the following conditions:

(a) The proposed amendment must be in writing and signed by at least five members of the Society.

(b) This signed proposal must be in the hands of the Secretary at least one month before the meeting of the Society at which it is to be considered.

(c) The Secretary shall mail copies of the proposed amendment to the members of the Society at least two weeks before the meeting.

## BY-LAWS

### DUES

(1) The annual dues for members or associate members, unless remitted or changed by the vote of the Society, shall be two dollars.

### SECRETARY

(2) The duties and privileges of the Secretary shall be as follows:

(a) He shall keep the records of the Society.

(b) Whenever the proper officers of a number of related societies shall have a conference with a view of determining a common time and place for the several annual meetings, he shall act as the delegate or representative of this Society. (See also 5.)

(c) He shall employ a typewriter or printer whenever in his judgment such employment will expedite the business of the Society, and

(d) He shall be reimbursed out of the funds of the Society for expenses incurred in attending meetings of the Society.

### TREASURER

(3) The duties and privileges of the Treasurer shall be as follows:

(a) He shall be in charge of the funds of the Society.

(b) At the Annual Business Meeting of the Society he shall present a statement to date of the funds of the Society.

(c) He shall employ a typewriter or printer whenever in his judgment such employment will expedite the business of the Society.

### AUDITING COMMITTEE

(4) The President shall annually appoint an auditing committee of two, who shall audit and report upon the financial record and statement of the Treasurer at the meeting for which they were appointed.

(5) The National Research Council allows the Society three representatives on the Division of Biology and Agriculture. Of these three representatives, one shall be elected each year to serve three years. The method of election shall be the same as that used in the election of the officers of the Society.

### MEETINGS

(6) It shall be the policy of the Society to hold meetings in both Eastern and Central-Western territory, and the distribution of the meetings between the two territories shall be determined in general on the basis of the representation of Eastern and Western members in the Society. See also 2-b.

### PROGRAM RULES

(7) In matters relating to programs for annual meetings the following rules shall be observed:

(a) Papers shall be listed and presented according to subject matter in the following groups: 1. Comparative Anatomy; 2. Embryology; 3. Cytology; 4. Genetics; 5. Comparative and General Physiology; 6. Ecology, and 7. Miscellaneous, or other groups at the discretion of the Secretary.

(b) Whenever conditions require it the Executive Committee shall schedule two or more groups for the same hour and rearrange the program to bring together papers on subjects of more general interest for meetings of the whole Society. The Committee, however, is instructed to avoid conflicts as much as possible.

(c) Papers shall be listed in their respective groups in the order received. Each active member is entitled to fifteen minutes of the time of the Society at the annual meeting which he may use to present one or more papers personally, or he may use any or all of this time in introducing papers of non-members on the program. The Society recognizes, however, the right of the Genetics Section to make their own program rules in conjunction with the Genetics Section of the Botanical Society of America.

(d) All papers not read when called for as listed shall be placed at the end of the group list, and, if not read when called for the second time, they shall be read by title only.

(e) The titles of ''introduced'' papers shall be listed in the groups after the titles of papers to be read by members. Such papers shall be read by title only in case the entire program cannot be completed during four regular sessions for reading papers.

(f) Fifteen minutes shall be the maximum time allowed for the presentation of a paper.

(g) Abstracts of papers for publication in the proceedings of the Society must be handed to the Secretary or his representative before final adjournment of the annual meeting.

### ASSOCIATE MEMBERS

(8) Associate members may become members of sections of the Society and are entitled to the journal privileges of members.

### HISTORICAL REVIEW

A review of the historical antecedents of the present American Society of Zoölogists will be found in *The Anatomical Record* for January, 1917. The list of officers and meeting places of the present Society found in the same place is brought up to date and reprinted here.

## LIST OF FORMER OFFICERS

### American Morphological Society

| President | Vice-President | Secretary-Treasurer |
|---|---|---|
| 1890—E. B. Wilson | ............ | J. P. McMurrich |
| 1891—C. O. Whitman | E. L. Mark | J. P. McMurrich |
| 1892—C. O. Whitman | H. F. Osborn | J. P. McMurrich |
| 1893—C. O. Whitman | E. B. Wilson | J. P. McMurrich |
| 1894—C. O. Whitman | W. B. Scott | G. H. Parker |
| 1895—E. B. Wilson | W. B. Scott | G. H. Parker |
| 1896—E. L. Mark | H. F. Osborn | G. H. Parker |
| 1897—C. S. Minot | S. I. Smith | G. H. Parker |
| 1898—H. F. Osborn | T. H. Morgan | G. H. Parker |
| 1899—E. G. Conklin | W. M. Wheeler | Bashford Dean |
| 1900—T. H. Morgan | H. C. Bumpus | J. S. Kingsley |
| 1901—J. S. Kingsley | E. A. Andrews | T. H. Montgomery |
| 1902—H. C. Bumpus | G. H. Parker | M. M. Metcalf |

*Additional Members of the Executive Committee*

1891—E. B. Wilson
H. F. Osborn
1892—E. L. Mark
T. H. Morgan
1893—T. H. Morgan
C. B. Davenport
1894—E. A. Andrews
F. H. Herrick
1895—T. H. Morgan
S. Watase
1896—E. G. Conklin
William Patten
1897—J. S. Kingsley
Bashford Dean
1898—C. B. Davenport
F. R. Lillie
1899—J. P. McMurrich
G. H. Parker
1900—F. R. Lillie
Jacob Reighard
1901—C. F. W. McClure
C. W. Hargitt
1902—H. S. Jennings
R. G. Harrison

### American Society of Zoölogists

| EASTERN BRANCH | President | CENTRAL BRANCH |
|---|---|---|
| G. H. Parker | 1903 | Jacob Reighard |
| E. A. Andrews | 1904 | C. H. Eigenmann |
| W. E. Castle | 1905 | F. R. Lillie |
| W. E. Castle | 1906 | C. C. Nutting |
| C. B. Davenport | 1907 | S. A. Forbes |
| W. M. Wheeler | 1908 | E. A. Birge |
| H. S. Jennings | 1909 | E. A. Birge |
| T. H. Montgomery | 1910 | C. E. McClung |
| H. V. Wilson | 1911 | George Lefevre |
| A. G. Mayer | 1912 | H. B. Ward |
| Raymond Pearl | 1913 | H. B. Ward |

## LIST OF FORMER OFFICERS

| EASTERN BRANCH | *Vice-President* | CENTRAL BRANCH |
|---|---|---|
| Jacob Reighard | 1903 | H. F. Nachtrieb |
| W. E. Castle | 1904 | S. J. Holmes |
| William Patten | 1905 | William A. Locy |
| William Patten | 1906 | George Lefevre |
| F. H. Herrick | 1907 | H. B. Ward |
| H. S. Jennings | 1908 | M. F. Guyer |
| H. V. Wilson | 1909 | M. F. Guyer |
| H. H. Wilder | 1910 | H. F. Nachtrieb |
| H. E. Crampton | 1911 | R. H. Walcott |
| G. A. Drew | 1912 | C. M. Child |
| Alex. Petrunkevitch | 1913 | C. M. Child |

*Secretary-Treasurer*

| | | |
|---|---|---|
| G. A. Drew | 1903 | Frank Smith |
| G. A. Drew | 1904 | F. R. Lillie |
| H. S. Pratt | 1905 | C. E. McClung |
| H. S. Pratt | 1906 | T. G. Lee |
| C. J. Herrick | 1907 | T. G. Lee |
| L. L. Woodruff | 1908 | T. G. Lee |
| L. L. Woodruff | 1909 | Charles Zeleny |
| H. W. Rand | 1910 | H. V. Neal |
| Raymond Pearl | 1911 | H. V. Neal |
| J. H. Gerould | 1912 | W. C. Curtis |
| Caswell Grave | 1913 | W. C. Curtis |

*Executive Committeemen*

| | |
|---|---|
| F. R. Lillie | George Lefevre |
| T. H. Montgomery | T. G. Lee |
| H. C. Bumpus | Herbert Osborn |
| H. S. Jennings | C. H. Eigenmann |
| E. A. Andrews | J. G. Needham |
| W. R. Coe | S. J. Holmes |
| G. A. Drew | W. A. Locy |
| M. M. Metcalf | C. M. Child |
| D. H. Tennent | R. H. Walcott |
| R. G. Harrison | W. C. Curtis |
| H. E. Jordan | Oscar Riddle |
| C. E. McClung | H. B. Ward |
| | Chauncey Juday |
| | H. W. Norris |
| | C. E. McClung |
| | H. F. Nachtrieb |

## American Society of Zoölogists (Amalgamated)

| | *President* | *Vice-President* | *Executive Committeemen* |
|---|---|---|---|
| 1914. | C. E. McClung | M. F. Guyer | H. E. Jordan—1 year<br>H. F. Nachtrieb—2 years<br>H. V. Wilson—3 years<br>George Lefevre—4 years<br>A. F. Shull—5 years |
| 1915. | W. A. Locy | W. E. Ritter | D. H. Tennent |
| 1916. | D. H. Tennent | Charles Zeleny | R. P. Bigelow—5 years<br>L. J. Cole—4 years |
| 1917. | M. M. Metcalf | Charles Zeleny | H. V. Wilson |
| 1918. | George Lefevre | L. L. Woodruff | M. M. Metcalf |
| 1919. | C. M. Child | H. H. Wilder | George Lefevre |
| 1920. | Gilman A. Drew | Caswell Grave | C. M. Child |
| 1921. | Charles A. Kofoid | Aaron L. Treadwell | G. A. Drew |
| 1922. | H. H. Wilder | Bennet M. Allen | C. A. Kofoid |
| 1923. | M. F. Guyer | Robert A. Budington | H. H. Wilder |
| 1924. | R. G. Harrison | R. K. Nabours | M. F. Guyer |
| 1925. | C. R. Stockard | C. R. Moore | R. G. Harrison |
| 1926. | S. O. Mast | W. C. Allee | C. R. Stockard |
| 1927. | S. J. Holmes | Robert Chambers, Jr. | S. O. Mast |

*Secretary-Treasurer*

1914–1918. Caswell Grave
1918–1921. W. C. Allee

| *Secretary* | *Treasurer* |
|---|---|
| 1921–1924. W. C. Allee | 1922. D. H. Tennent |
| 1925. D. E. Minnich | 1923–1924. R. H. Bowen |
| | 1925. L. B. Arey |

*Representative in the Division of Biology and Agriculture, National Research Council*

| | | |
|---|---|---|
| M. F. Guyer 1919–1921 | G. H. Parker 1919–1922 | F. R. Lillie 1919–1923 |
| William Patten 1921–1924 | H. S. Jennings 1922–1925 | E. G. Conklin 1923–1926 |
| J. T. Patterson 1924–1927 | L. L. Woodruff 1925–1928 | |

*Associate Editors of the Journal of Morphology*

| | | | |
|---|---|---|---|
| 1921. | Gary N. Calkins | J. S. Kingsley | William Patten |
| 1921–1922. | E. G. Conklin | M. F. Guyer | W. M. Wheeler |
| 1921–1923. | C. A. Kofoid | F. R. Lillie | J. T. Patterson |
| 1922–1924. | G. A. Drew | H. V. Neal | L. L. Woodruff |
| 1923–1925. | Caswell Grave | D. H. Tennent | H. V. Wilson |
| 1924–1926. | Helen Dean King | S. O. Mast | A. A. Schaeffer |
| 1925–1928. | R. W. Hegner | Fernandus Payne | H. B. Ward |

## LIST OF PLACES OF MEETING

### American Morphological Society

1890—Boston
1891—Philadelphia
1892—Princeton
1893—New Haven
1894—Baltimore
1895—Philadelphia
1896—Boston
1897—Ithaca
1898—New York
1899—New Haven
1900—Baltimore
1901—Chicago
1902—Washington

### Central Naturalists

1899—Chicago
1900—Chicago

### Society of American Zoölogists

1901—Chicago
1902—Washington

### American Society of Zoölogists

#### EASTERN BRANCH

1903—Philadelphia
1904—Philadelphia
1906—New York
1907—New Haven
1909—Boston
1910—Ithaca

#### JOINT MEETINGS

1905—Ann Arbor
1908—Baltimore
1911—Princeton
1912—Cleveland
1913—Philadelphia

#### CENTRAL BRANCH

1903—St. Louis
1905—(Mch.) Chicago
1907—(Mch.) Madison
1907—Chicago
1910—(Apr.) Iowa City
1910—Minneapolis
1912—(Apr.) Urbana

#### MEETING PLACES

1914—Philadelphia
1915—Columbus
1916—New York
1917—Minneapolis
1918—Baltimore
1919—St. Louis
1920—Chicago
1921—Toronto
1922—Boston
1923—Cincinnati
1924—Washington
1925—New Haven
1926—Philadelphia
1927—Nashville

# AMERICAN SOCIETY OF ZOÖLOGISTS

## OFFICERS AND LIST OF MEMBERS

### *Officers for 1928*

| | |
|---|---|
| *President* | CASWELL GRAVE |
| *Vice-President* | M. H. JACOBS |
| *Secretary* | D. E. MINNICH |
| *Treasurer* | L. B. AREY |

### *Executive Committee*

| | |
|---|---|
| M. F. GUYER | 1928 |
| R. G. HARRISON | 1929 |
| C. R. STOCKARD | 1930 |
| S. O. MAST | 1931 |
| S. J. HOLMES | 1932 |

### *Officers of Genetics Section*

| | |
|---|---|
| *Chairman* | H. J. MULLER |
| *Secretary-Treasurer* | L. C. DUNN |
| *Society Representative* | O. E. WHITE |
| *Representative on Editorial Board of "Journal of Heredity"* | M. DEMEREĆ |
| *Representative on "Biological Abstracts"* | F. B. HANSON |

### *Representatives of the Society in the Division of Biology and Agriculture of the National Research Council*

| | |
|---|---|
| G. H. PARKER | 1929 |
| L. C. DUNN, Alternate | 1929 |

### *Representative of the Society on the Type Culture Committee of the National Research Council*

| | |
|---|---|
| C. A. KOFOID | 1929 |

### *Representatives of the Society on the Council of The American Association for the Advancement of Science*

W. C. ALLEE　　F. E. LUTZ

### *Representatives of the Society on the Council of the Union of American Biological Societies*

ROSS G. HARRISON　　D. E. MINNICH

### *Representatives of the Society on the Advisory Editorial Board of "Biological Abstracts"*

| | |
|---|---|
| H. S. PRATT, G. K. NOBLE | *Taxonomy* |
| D. H. TENNENT | *General Embryology* |
| M. H. JACOBS | *General Physiology* |
| R. H. BOWEN | *Cytology* |

## EDITORIAL BOARD OF THE JOURNAL OF MORPHOLOGY AND PHYSIOLOGY

*Managing Editor* (term expires 1931)........................C. E. McClung

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| | |
|---|---|
| To serve until 1929.................................. | M. H. Jacobs<br>L. J. Cole<br>J. H. Gerould |
| To serve until 1930.................................. | J. Percy Moore<br>W. C. Curtis<br>A. F. Shull |
| To serve until 1931.................................. | Edgar Allen<br>G. A. Baitsell<br>R. E. Coker |

## LIFE AND ACTIVE MEMBERS

(Life members indicated by an asterisk)

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# FACTORS CONTROLLING THE ARTERIAL SUPPLY OF THE TESTIS UNDER EXPERIMENTAL CONDITIONS

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ONE TEXT FIGURE AND THREE PLATES (SIX FIGURES)

The presence of well-defined anastomoses between the three arteries of the testis at the cauda epididymidis has been repeatedly demonstrated by morphological studies. The testicular atrophy, however, that has followed experimental and clinical ligation of the internal spermatic vessels indicates the need of an investigation of factors controlling the sufficiency of these arterial anastomoses.

In 1889, Jarisch gave a complete account of the arterial supply of the human testis, which was considered authoritative and quoted for many years in anatomical texts. He established the fact that the testis receives its arterial supply from three sources—the internal spermatic, the deferential, and the external spermatic arteries, the main supply coming from the internal spermatic (compare fig. 1). He observed that the internal spermatic artery, upon entering the spermatic cord, divides into two or three tortuous branches. The upper of these usually supplies the head of the epididymis, while the others enter the mediastinum to supply the testis. Another branch, arising either from the arteria capitis epididymidis or directly from the internal spermatic, supplies the body of the epididymis, and then at the tail of the epididymis enters into anastomosis with the deferential and the external spermatic arteries. Jarisch maintained that the deferential artery is not given off from the hypogastric artery, but from the root of the obliterated umbilical artery,

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and follows a tortuous course to supply the ductus deferens and the tail of the epididymis. He described the external spermatic artery as a branch of the inferior epigastric, supplying the coverings of the testis and the spermatic cord.

Capurro ('01) studied the arterial anastomoses of the testis in the dog by injecting gelatin into the internal spermatic artery through the aorta. He demonstrated by subsequent dissection that the deferential artery communicates with the epididymal branches of the internal spermatic through terminal rami and through the intermediation of a rich arterial network, and that this anastomosis is united with the terminal rami of the external spermatic artery. He showed, further, that the scrotal arteries of one side communicate across the midline with those of the other side, and that an anastomosis exists between the scrotal arteries and the terminal rami of the external spermatic.

Somewhat later ('09), these observations upon the arterial anastomoses were verified by Picque and Worms, who injected the internal spermatic artery directly at its entrance into the spermatic cord. They found that the mode of communication between the three arteries of the testis at the cauda epididymidis is subject to frequent variation.

As contrasted with the arterial supply, the venous circulation of the testis and epididymis was not worked out until 1898, when Haberer pointed out that the blood is returned by four possible routes. According to his investigation, the blood from the testis passes into the anterior and posterior parts of the pampiniform plexus; that from the upper two-thirds of the epididymis reaches the anterior pampiniform plexus by way of the epididymal veins, while that from the tail of the epididymis is carried by the deferential vein into the venous plexus about the seminal vesicles. The blood from the coverings of the testis and spermatic cord is returned by the external spermatic vein into the inferior epigastric vein.

In 1909, Hill made a special study of the arrangement of the vessels beyond the mediastinum in the human testis, employing injection, clearing, and corrosion methods; our present conception of this arrangement is based almost entirely upon his work. It is an outstanding fact that, aside from the description of the tunica vasculosa by Astley Cooper and the incomplete descriptions of the arteries and veins in the parenchyma of the testis by Jarisch and Haberer, no real contribution on the finer distribution of the vessels had been made prior to that of Hill.

The first experimental approach to the problem of arterial anastomoses of the testis was made by Astley Cooper, in 1845, who studied the effect of ligation of the internal spermatic artery and vein, and observed that it was followed by sloughing and gangrene of the testis. Miflet ('79) concluded that obliteration of the internal spermatic artery was followed by hemorrhagic infarction of the testis, and that ligation of the internal spermatic veins alone was sufficient to cause degeneration of the testicular parenchyma. He regarded the internal spermatic artery as an end artery. The opposite view, that obliteration of the internal spermatic vessels causes neither sloughing nor atrophy, was later held by Kocher ('87), Treves ('91), and Bennett ('93).

In 1895, Griffiths revived the older view and observed that obliteration of the internal spermatic artery led to a diminution in the size of the testis with degenerative changes in the seminal tubules, and that ligation of the pampiniform plexus led to swelling of the testis with final atrophy of the parenchyma. Sloughing and complete atrophy followed interruption of both the internal spermatic artery and the pampiniform plexus.

After a marked disagreement among investigators on the fate of the testis after ligation of its blood vessels, Wangensteen finally, in 1927, arrived at conclusions similar to those of Griffiths. He maintained that statements to the effect that ligation of the internal spermatic artery and pampiniform plexus is without deleterious effect on the testis will not bear investigation.

Clinical observations upon the question of ligation were made by Mignon in 1902 and independently by Bevan the following year. Both maintained that all the vessels in the spermatic cord, with the exception of those accompanying the ductus deferens, could be sacrificed without producing any visible nutritional disturbance in the testis. This view found its practical application in orchidopexy for incomplete descent, and section of these vessels was advocated by them as a measure to aid low fixation of the testis in the scrotum.

Mixter ('24), in a report on the results of operations for undescended testis, noted that it was necessary to section the internal spermatic vessels in fifteen out of 107 cases, and that atrophy of the testis followed ligation in all but two cases. Occasionally, atrophy occurred when the vessels were not divided, and this result he attributed to the accidental injury of the vessels during operation.

Though it is generally assumed that sufficient blood for nutrition reaches the testis through the arterial anastomoses after ligation of the internal spermatic vessels, it appears that no satisfactory explanation has ever been given for the occurrence of atrophy of the testis following ligation of these vessels.

In the course of some preliminary experiments in ligation, we were greatly impressed with the observation that, while the testis atrophied, the epididymis remained normal after interruption of the internal spermatic vessels. This fact had previously been observed by Miflet ('79) and Wangensteen ('27). In seeking an explanation for this difference, our attention was drawn to the fact that the portion of the tunica albuginea which covers the epididymis is thinner and less resistant than the part which covers the testis. This suggested to us the idea that the congestion in the testis which follows ligation of the vessels may be due to the unyielding nature of its fibrous investment.

In addition, our attention was attracted to a characteristic reaction associated with ligation of the internal spermatic vessels, namely, the sudden appearance of firmness (or so-called 'stone-hardness') in the testis—an increased tension which is remarkably constant in different individuals. It became our objective to duplicate this stone-hardness experimentally and to test under this condition the degree of penetration of injection media. Incidentally, the methods employed made it possible to display the arrangement and caliber of the arterial anastomoses at the cauda epididymidis.

### METHOD OF INVESTIGATION

The fundamental feature in the experiments undertaken consisted in the production of different degrees of tension in the testis through saline perfusions and the subsequent injection of the organ with solutions of celloidin and Berlin blue. The injections were controlled and the specimens prepared for study in accordance with the methods of Morison ('23). In order to obtain complete penetration of the arterial system of the testis, it was found necessary to use a solution of celloidin lower in viscosity than that used in renal work, and this necessity was apparently due to the unusual length and tortuosity of the internal spermatic artery. A solution, colored with alkanin, of the strength given in the following formula, insures complete penetration and possesses a viscosity high enough to prevent capillary penetration:

| | |
|---|---|
| Parlodion, | 1.25 gram |
| Camphor, | 0.80 gram |
| Acetone, | 100.00 cc. |

The present study consists of three series of experiments confined exclusively to dogs. In all cases the posterior abdominal wall was exposed under ether anaesthesia through a low midline incision in the anterior abdominal wall. The aortic segment corresponding to the origin of the internal spermatic artery was used as the site of injection, since its thick wall is well adapted to withstand the bulging and distortion that invariably occurs in thinner vessels under high pressure. The lumbar arteries arising from the segment were ligated to prevent the celloidin from reaching the deferential and cremasteric arteries by other routes than the anastomotic channels. After the animal was killed and submerged in water, the vascular system of the testis was washed free of blood by perfusing with physiological salt solution.

With a view to maintaining normal tension in the first series of eight dogs, the time of the saline perfusion was limited to four minutes, and the testes were gently and continuously massaged during the perfusion to assist the flow through the venous system. Normal tension, as employed in this series, was the tension evident upon palpation of a normal living testis. To duplicate the 'stone-hardness' found in the living testis after ligation of the internal spermatic vessels, the perfusion in the second series of eight dogs was continued for a period of about twenty minutes. This tension was taken as the standard of increased tension and determined by palpation.

The time consumed in preparing the animal for perfusion varied from thirty to forty-five minutes and the pressure necessary to start the saline circulation ranged from 80 to 120 mm. of mercury. It was found that this initial pressure was directly proportional to the time spent in the preparation of the animal.

In the preceding two series of animals, celloidin was employed as the injection medium and introduced with a pressure of 500 mm. of mercury for twenty-four hours. The tissues surrounding the testis were then dissected away, and the spermatic cord and testis were removed en masse. The testes from the first five dogs in these two series were placed in 75 per cent hydrochloric acid for twenty-four hours, and those of the last three dogs of these series were preserved for minute dissection with binocular magnifiers.

In a third series of four dogs, normal tension was maintained in the first two and increased tension in the other two animals. A 2 per cent solution of Berlin blue in distilled water was employed for injection and introduced with a pressure of 120 mm. of mercury for twenty minutes. All the testes in the dogs of this last series were subjected to minute dissection.

## OBSERVATIONS

### *First series of testes: celloidin injections; normal tension*

In limiting the saline perfusion to four minutes, normal tension was maintained in both testes in six dogs and in the left testis only, in two dogs; the right testis in the latter two animals was excluded from the series, inasmuch as the tension increased above the normal. The distribution of the celloidin during the course of the injection was carefully noted. It passed from the isolated aortic segment into the peritoneal branches of the internal spermatic artery, and also into the small arteries of the renal capsule by way of anastomosing channels; in three specimens of the series the injection extended into and filled the renal artery. The celloidin also advanced downward in the internal spermatic artery, and as it was filling the vessels of the testicular capsule, it ascended simultaneously in the deferential and cremasteric arteries. Subsequent dissection showed that the celloidin passed from the deferential into the hypogastric artery with its branches, and from the cremasteric into the inferior epigastric and the external iliac, passing upward from the latter to meet the injection mass from the deferential, and downward to enter the femoral artery and its branches.

The arterial anastomoses in the testis of the dog and their general relation are indicated diagrammatically in figure 1.

The casts produced by corrosion show that the celloidin extended into the finest radicles of the arterial system of the testis and the epididymis (figs. 3 and 4). The tortuous course of the internal spermatic artery in the distal part of the cord and the division of this artery at the mediastinum into rami penetrating the lobules are well defined in the figure. The capsular artery, arising at the hilus, proceeds downward and then forward to almost completely encircle the testis, anastomosing at its termination with rami of the internal spermatic at the upper pole. Some of the rami of the internal spermatic artery given off at the mediastinum were broken off from the cast in the process of corrosion, but the preserved branches can be seen penetrating the lobular spaces to join the rami from the capsular artery. The origin of the epididymal artery is not shown in the figure, but it arose usually from the internal spermatic artery above its convoluted portion.

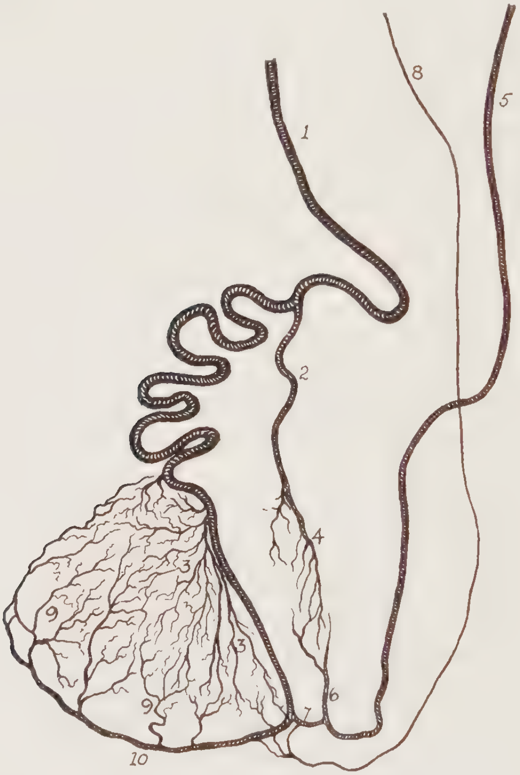

Fig. 1 Diagram of the three arteries of the testis in the dog, showing the mode of distribution and termination of these vessels. The diagram is based upon a study of the celloidin casts derived from this experiment. *1,* internal spermatic artery; *2,* epididymal artery; *3,* mediastinal rami penetrating the lobules; *4,* terminal part of epididymal artery anastomosing with deferential artery; *5* and *6,* deferential artery; *7,* anastomosis between capsular and deferential arteries; *8,* external spermatic artery; *9,* rami from capsular artery penetrating lobules and anastomosing with mediastinal rami; *10,* capsular artery.

Only the terminal portion of the deferential artery has been preserved, together with some of its minute twigs going to supply the ductus deferens. The capsular artery gives off a branch (deep anastomosing ramus) that proceeds posterolaterally to unite with the terminal loop of the deferential, tapering toward its termination. This ramus and the capsular artery subtend an angle in which is lodged a fine vascular network, from which the surrounding tissue has only in part been corroded. This retiform structure connects the cremasteric artery with the internal spermatic system, and only the stump of the terminal ramus of the cremasteric remains intact in the figure. The spermaticocremasteric anastomosis was difficult to preserve in most specimens, owing to the delicacy of structure and lack of tissue support. The spermaticodeferential and spermaticocremasteric anastomoses showed considerable variation in the casts of this series, but the variations conformed in a general way to the three types distinguished by Picque and Worms.

Upon dissection under the binocular magnifiers, it was demonstrated that the three arteries in the spermatic cord give off numerous small rami, which freely anastomose with each other. In two specimens the celloidin passed from the cremasteric artery through connecting channels to the anterior and posterior scrotal arteries. The external pudendal and the perineal arteries supply, respectively, the anterior and posterior parts of the scrotum, and a communication exists in this cutaneous plane across the raphé—showing that the arterial anastomosis about the testis is an extensive system.

### *Second series of testes: celloidin injections; increased tension*

In permitting the saline perfusion to continue for twenty minutes, the standard of increased tension was attained in both testes in three dogs and in the right testes only, in five dogs. The hardening occurred slowly and gradually upon the introduction of the saline; massage with compression of the testis produced no softening after the tension once began to increase. No satisfactory explanation can be offered for the failure of the left testes in five dogs to harden. The manometric pressure during the saline perfusion of the latter was increased to 300 mm. of mercury without obtaining the experimental standard of increased tension. The tension increased, however, in these testes to values lying between the normal and the standard of increased tension, and subsequent penetration of the celloidin was in inverse proportion to the amount of tension. In the eleven testes which reached the standard of increased tension, the epididymis remained in each instance considerably softer than the testicular portion.

The celloidin casts of the series were incomplete and reproduced only the larger rami of the intracapsular arterial network. The pronounced effect of increased tension upon the penetration of the celloidin, in contrast to that under normal tension, is shown in figure 2. The epididymal artery, arising from the upper convoluted portion of the internal spermatic, is seen to divide immediately above the epididymis into its terminal branches. An ascending ramus from the capsular artery (*3*, fig. 2) has been displaced downward to show the absence of the finer arterial network within the capsule. On the other hand, the penetration in the epididymis, though incomplete in this particular case, was under the same conditions surprisingly complete under increased tension in several specimens of this group. Six casts demonstrate the presence of a fine arterial network between the terminal rami of the epididymal artery.

The deferential artery was completely injected in six testes of the group, the injection was incomplete in three, and in the remaining two the celloidin failed altogether to reach the artery. The cremasteric artery, however, was completely injected in ten of the testes.

With reference to the cause of this gradual increase in tension in the eleven testes of the group, it is quite possible that this is due to an extravasation and infiltration of the salt solution into the tissues. Hill ('09) observed, while perfusing the human testis with a pressure of 60 to 80 mm. of mercury, that the gland became firm, and this phenomenon he believed to be due to the passage of fluid between the endothelial cells of the capillaries into adjacent tissues. The occurrence of an extravasation with a pressure below the normal systolic blood pressure was ascribed by him to the rapid death of the endothelial cells.

### *Third series of testes: Berlin-blue injections*

The penetration of the solution of Berlin blue in the testis and in the epididymis under normal and increased tension was similar to that of the celloidin under the same conditions. Under normal tension the dye permeated both testis and epididymis uniformly (fig. 6). In the organ injected under increased tension, as shown in figure 7, the Berlin blue penetrated the testis to a depth of only 1 mm., while in the epididymis, under the same conditions, the injection was complete. The passage of the Berlin blue through the anastomotic channels in the spermatic cord and at the tail of the epididymis was similar to that of the celloidin.

## DISCUSSION

The outstanding result of this investigation is the difference in the degree of penetration of celloidin and Berlin blue in the testis and in the epididymis under increased tension. During increased tension in these organs, as produced by saline perfusions, the penetration of the injection within the capsule of the testis was very slight, whereas in the epididymis the injection was quite complete. During the saline perfusions the testis usually became tenser than the epididymis.

It is a notable fact, as originally shown by Miflet and Wangensteen, that, after experimental ligation of the internal spermatic vessels, the testis atrophies while the epididymis remains normal. We have conducted a series of ligations in which the observations of these investigators were confirmed. In the course of our experiments it was further established that the tension after ligation was uniformly greater in the testis than in the epididymis.

The present results demonstrate that pressure disturbances occur within the testis after prolonged saline perfusions and that comparable disturbances occur in the testis after ligation of the internal spermatic vessels. In our experimental injections this disturbance was measured by the penetration of either celloidin or Berlin blue; in experimental ligation it is determined by the degree of atrophy. The extent to which the degree of penetration in experimental injections is equivalent to the degree of atrophy following ligation remains a problem for further investigation.

The pronounced difference in the behavior of the testis and of the epididymis toward injections under increased tension and toward experimental ligation of the internal spermatic vessels appears to be due to the fact that the portion of the tunica albuginea overlying the testis is thicker and more unyielding than that covering the epididymis (fig. 5). If pressure within the capsule after ligation of the internal spermatic vessels is a factor in the production of atrophy in the testis, it is possible that either an anterior longitudinal incision through the tunica albuginea or a bisection of the testis with an orchidorrhaphy would prevent atrophy. The results of a series of experiments now being conducted with a view to testing this hypothesis will be given in a subsequent report.

## SUMMARY

The 'stone-hardness' following interruption of the internal spermatic vessels in the living testis has been duplicated in the dead testis by prolonged perfusions of physiologic salt solution.

The testis invariably became tenser than the epididymis when both organs were perfused with salt solution for twenty minutes through the internal spermatic artery.

After both organs were thus subjected to increased tension in the same circuit, the penetration of celloidin and Berlin blue was complete in the epididymis, but very incomplete in the testis.

Testicular penetration of the injections was inversely proportional to variations in testicular tension.

The difference in behavior of the testis and the epididymis toward experimental injections corresponds to the results obtained in experimental ligation of the internal spermatic vessels.

It is suggested that this difference in behavior of the testis and the epididymis is due to the difference in structure of the enveloping capsule, which is thin and yielding over the epididymis, but thick and resistant over the testis.

This hypothesis is being tested by a series of experimental ligations, each of which is supplemented by an anterior longitudinal incision through the tunica albuginea or a bisection of the testis with an orchidorrhaphy.

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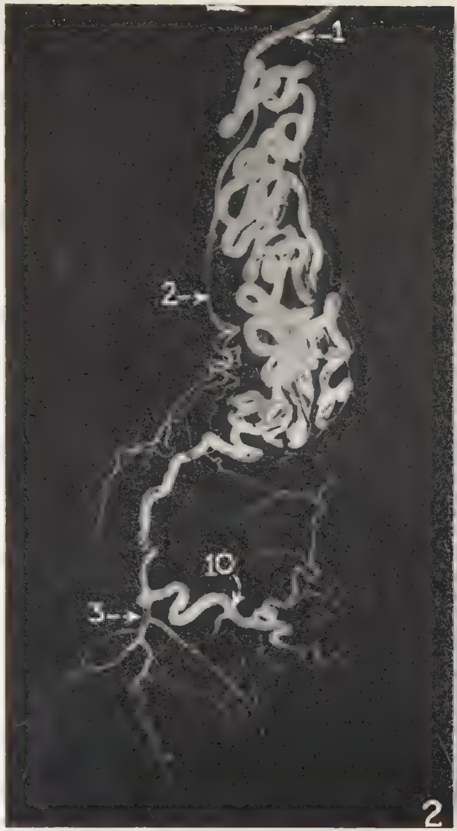
# PLATES

## PLATE 1

### EXPLANATION OF FIGURES

Photographs of two celloidin casts of the arterial system of the right testis in the dog. The testis in figure 2 was allowed to harden, while the one in figure 3 was permitted to remain normal in tension during the perfusion with salt solution. *1*, internal spermatic artery; *2*, epididymal artery; *3*, mediastinal rami penetrating the lobules; *4*, epididymis; *5*, deferential artery; *6*, terminal part of deferential artery entering the tail of epididymis; *7*, ramus from capsular artery anastomosing with deferential artery; *8*, terminal stump of cremasteric artery; *9*, rami from capsular artery penetrating the lobules; *10*, capsular artery; *11*, vascular network of spermaticocremasteric anastomosis.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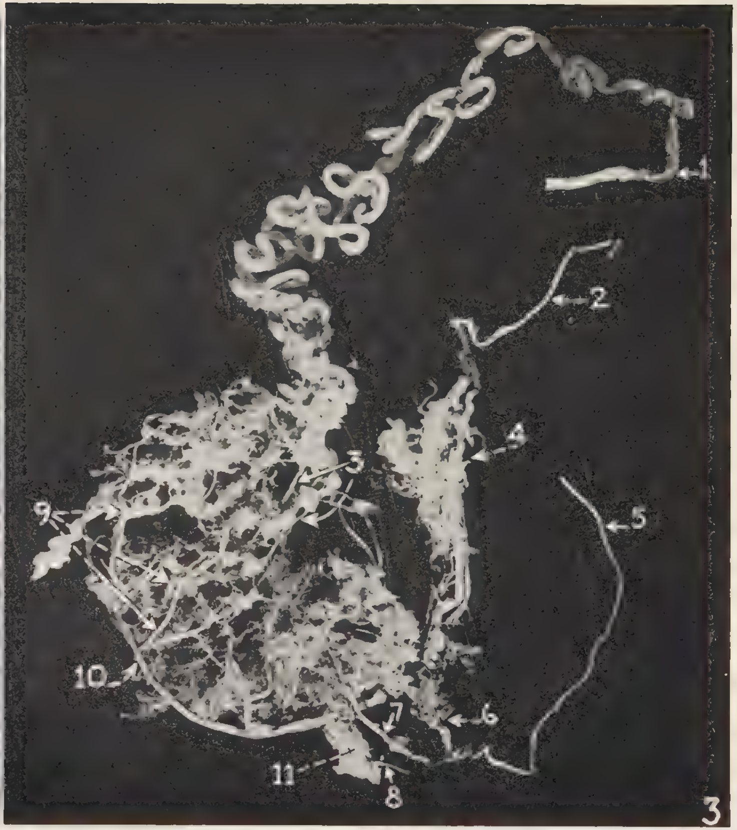
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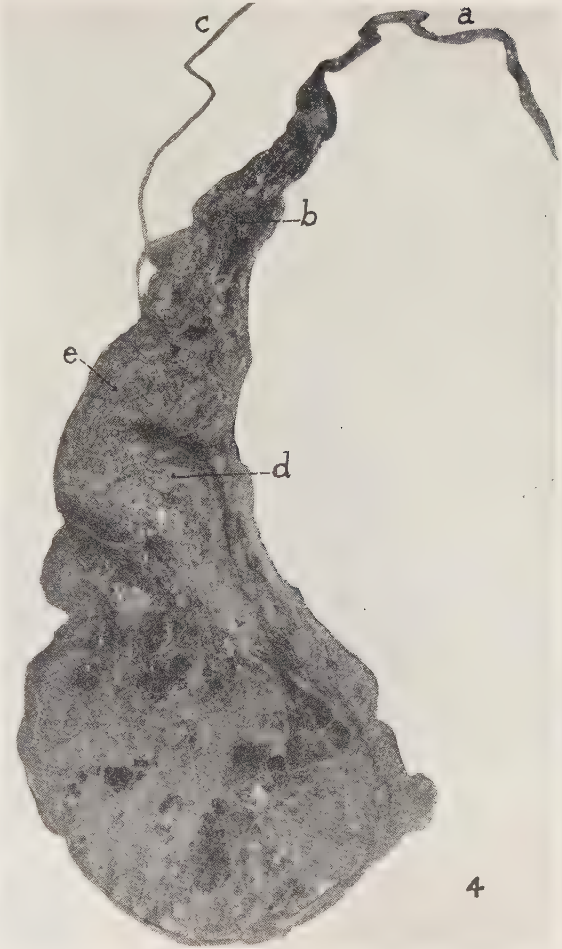
## PLATE 2

### EXPLANATION OF FIGURES

4 Photograph of the right testis of a dog injected with celloidin under normal tension and subjected to only partial corrosion. *a*, internal spermatic artery; *b* and *e*, pampiniform plexus surrounding convolutions of internal spermatic artery; *c*, deferential artery; *d*, lobule of the epididymis.

5 Photograph of a transverse section through the superior extremity of the testis of a dog, showing the difference in thickness of the tunica albuginea investing the testis proper and the epididymis. *t.a.epid.*, tunica albuginea of epididymis; *t.a.testis*, tunica albuginea of testis.



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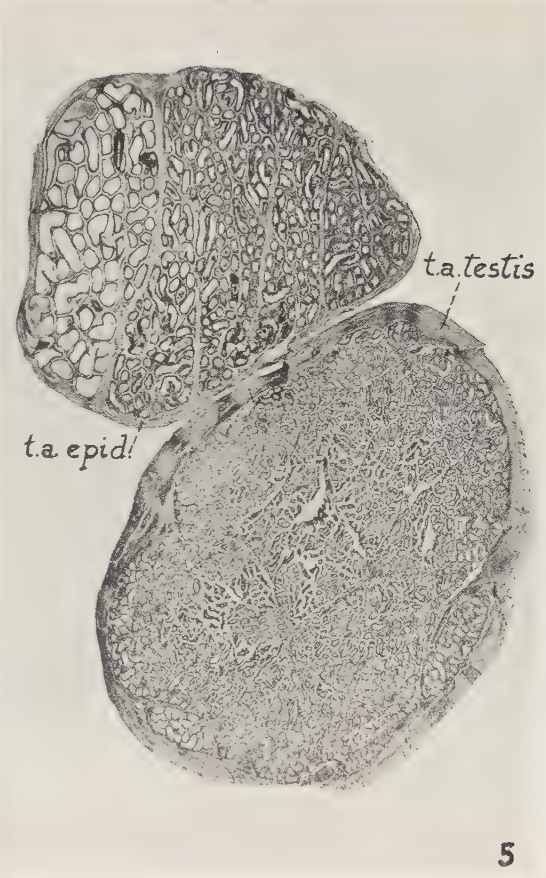
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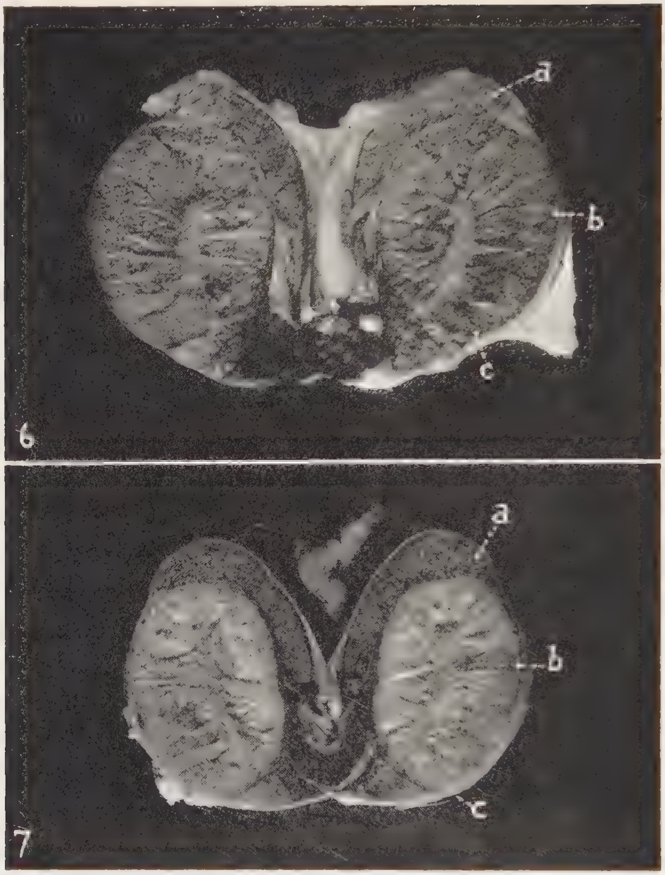

Photographs of two testes of the left side in a dog injected with an aqueous solution of Berlin blue. The testis in figure 6 was injected under normal tension, while the one in figure 7 was injected under increased tension. The testes are divided in the sagittal plane, slightly lateral to the midline of the organ, to show the extent of penetration of the dye in the testis proper and in the epididymis. The darker color in the photograph indicates penetration and the white color, an absence of penetration. *a*, caput epididymidis; *b*, anterior surface of the testis; *c*, inferior extremity of the testis.

# A NEW MOISTURE-CONSERVING DISSECTING TABLE

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SIX TEXT FIGURES

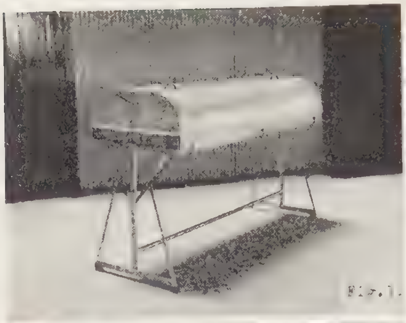
For several years the authors have been looking for a dissecting table which would answer the following:

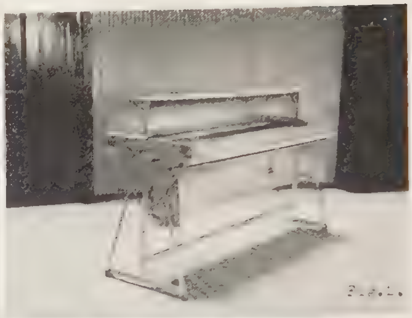
1. Keep the dissecting material moist and in good condition without the use of oiled coverings or grease.
2. Be substantial, free as possible from rust and breakage.
3. Be comfortable to work at, clean, neat in appearance, and not too costly.

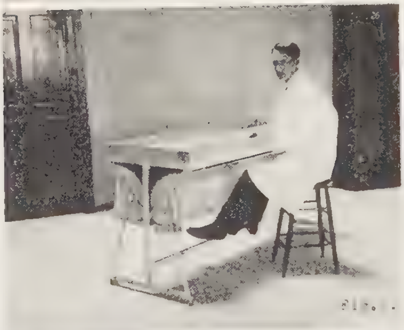
The accompanying photographs represent the results of our efforts. A sample table was used a year and, with minor changes, has been adopted for the entire medical class in anatomy.

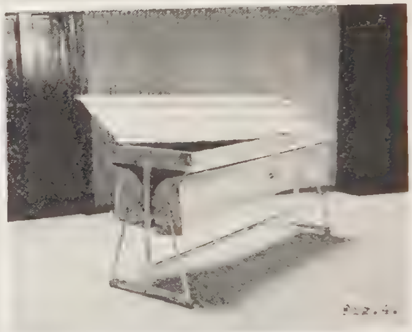
The table consists of five parts: a frame constructed largely of angle iron, a shallow pan of rustless iron (ascoloy), a removable tray of ascoloy within this pan, and two lids of galvanized iron hinged to the frame and swinging through an arc of 180°.

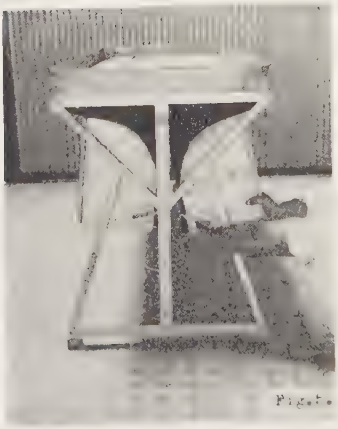
The frame is 27 inches wide and 77 inches long. The edge to which the cover is hinged is 31¼ inches from the floor. As seen in figures 2 to 5, the pan sets into the frame, but is not attached to it in any way. The pan is further supported along its midline by a T welded at its ends to the vertical supports of the end pieces (inside number 14 of the photographs). This median longitudinal T is further supported by a 1-inch strap-iron brace (fig. 1) welded to it and to the end piece. Malleable pipe 1½ inches outside, 7½ inches











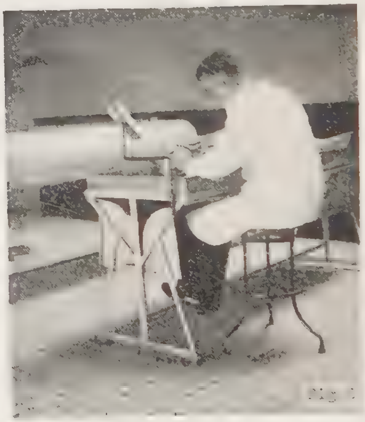


Fig. 1 Table closed.
Fig. 2 One lid closed, one open and hooked to frame.
Fig. 3 Table open, showing that adequate knee room is afforded.
Fig. 4 Table open, tray tilted up to show corrugations and holes.
Fig. 5 End view, showing method of hooking lids to frame.
Fig. 6 Table, showing bookrack for dissecting manual.

from the floor, forms a foot support and tie for the end pieces (fig. 3). Four hard-wood blocks act as feet, allowing the table to be pushed about without damage to the floor. The bracing of the end pieces, illustrated best in figure 5, gives great strength and rigidity to the frame.

The corrugated tray upon which the body lies is shown best in figure 4. This is made of 22-gauge ascoloy and fits close to the sides of the pan (fig. 5). The tray is supported by three transverse [’s 1½ inches high, made of heavy ascoloy, riveted to it. Three rows of holes in the tray allow moisture to rise from a weak formalin solution kept in the pan to the depth of an inch or so. This creates enough moisture in the closed table to keep the body in good shape.

The hinged lids were designed to make a nearly moisture-proof compartment when closed, and to swing out of the way, giving ample knee space for the dissector at work (fig. 3). Each lid swings on three hinges (fig. 1) and is hooked to the end pieces (figs. 4 and 5) when the table is open. When closed, one lid fits into the other by a tongue-and-groove joint. Each lid is strengthened by a 1-inch angle iron running lengthwise (figs. 1 and 2). There are approximated, ⌋⌊, when the table is closed and clamped. A ⅜-inch hole is drilled through these angles near the middle for a padlock. As seen in figures 1 and 2, the ends of the covers rest on the rolled edge of the ascoloy pan, completing a tightly closed chamber 75 inches long, 25½ inches wide, and 12 inches high.

Recently, we have tried out several bookracks for holding the dissecting manual. The most satisfactory thus far is shown in figure 6. A socket is firmly attached to the frame. The bent rod rotates freely in this socket, and the bookholder itself also rotates on the upper end of the rod. This gives adequate adjustment for the comfort of the dissector and does not interfere with closing the table. Four bookholders of this type will be put on each table.

In conclusion, we wish to say that these tables were constructed in Louisville. To date they have proved very satisfactory, and we trust they may be of value in making the study of anatomy more efficient, not only here, but elsewhere.

# AN EXPERIMENTAL STUDY OF THE FAT-BODIES IN TRITURUS (DIEMYCTYLUS) VIRIDESCENS

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ELEVEN TEXT FIGURES AND ONE PLATE (EIGHT FIGURES)

## INTRODUCTION

Paired fat-bodies occur in all amphibians and in lizards. In the Anura they are digitate structures located at the anterior end of the genital organs; in urodeles they are characteristically band-like in shape, lie parallel to the kidneys, between them and the gonads and ventral to the latter organs. They usually appear to be composed of bright-yellow fat-globules, but occasionally are of a whitish hue. Their position in close association with the gonads hints at a possible interdependence of the two organs, while their essentially fatty nature suggests that they may act as a nutritional reserve for the body as a whole or a source from which the gonads derive energy. The latter hypothesis the writers have attempted to test in one species of urodeles, Triturus (Diemyctylus) viridescens, by removing the fat-bodies and by subjecting the animals to inanition experiments.

### *Origin of the fat-bodies*

A survey of the literature on fat-bodies indicates that two problems have attracted particular attention, the origin of these bodies and their function. The first of these has been investigated by examination of normal embryological material and by experimental methods, as a result of which two different conclusions have been reached. An independent origin of gonad and fat-body is the contention of one group of workers, while a second claims that a common ridge is the anlage of both.

Among those advocating the former idea is Giles ('89), who from a study of normal embryos was led to believe that the fat-body in the frog was the result of fatty degeneration of the pronephros. Giglio-Tos (Bouin, '99), emphasizing the fact that a degenerate organ does not function, claimed that the rudiment of the fat-body in frog embryos was not derived from a degenerating pronephros, but from the connective tissue of the adventitia of the posterior vena cava near the end of the genital ridge. Kennel ('12 b) located the earliest traces of the fat-bodies in an accumulation of fatty substances in the meshes of the serous membrane around certain peritoneal vessels leading to the vena cava.

The greater weight of evidence seems to lie with those who derive the fat-body and gonad from a common ridge. Von Wittich as early as 1853 (Giles, '89) stated that the fat-body differentiated from the anterior end of the genital ridge, while the posterior part of the ridge, separated by a constriction from its anterior section, became the definitive genital organ. Marshall and Bles ('90) also agreed with von Wittich and were able to demonstrate the error in Giles's results by showing that pronephros and fat-body were present simultaneously in frog embryos. In a more discriminating analysis of the problem, Bouin ('99) emphasized the fact that, while fat-body and genital gland originated from a common ridge formed by a peritoneal fold, the fat-body differentiated from the anterior part of this ridge where no primordial germ cells occurred. Moreover, he challenged the origin as set forth by Giglio-Tos because the adventitious wall of the inferior vena cava was not present at the time when the regions of this ridge could be distinguished by the presence of primordial germ cells in the posterior part.

In experiments dealing with the germ cells of urodeles, Humphrey ('27 a) has also touched upon this problem. In embryos of Amblystoma maculatum and jeffersonianum (stages 32 to 37) he removed that portion of the intermediate mesoderm which included the germ cells (seventh to the sixteenth somites), together with the immediately adjacent parts of the axial and lateral mesoderm, the pronephric duct, and the overlying ectoderm. Fat-bodies of variable size, but always subnormal, occurred on the operated side in 63 per cent of the animals that developed for more than thirty-five days after the operation; in about 80 per cent of these this fat-body was located in the cephalic half of the genital territory. The evidence suggests that the primordium of this organ is of greater cephalic extent than that of the gonad, since the removal of the mesodermal primordia of the gonad and fat-body was most often incomplete at the anterior end—resulting in the formation of a fat-body, but not of a gonad. In a second study ('27 b) Humphrey grafted the intermediate mesoderm of Amblystoma (including the structures listed above) into the somatopleure of other embryos of approximately the same age (stages 30 to 35). Seven embryos survived. In the host which lived the longest (forty-four days), a large gonad and associated fat-body resulted from the graft. In Humphrey's third series of experiments ('28) the intermediate mesoderm gave rise to a fat-body in one-half of the seventy-four grafts. This fat-body developed quite independently of the gonad in many cases. These experiments, especially the first in which a fat-body developed in the absence of the primordial germ cells, seemed to substantiate Bouin's idea of a common ridge. Thus, the sum total of histological and experimental evidence appears to point to the differentiation of the fat-body from the anterior part of the ridge, the posterior part of which gives rise to the genital organ, and emphasizes the association of fat-body and gonad in development.

## *Function of fat-bodies*

Opinions held by zoölogists as to the function of fat-bodies differ, but in all cases they are closely correlated with seasonal variations that have been observed in the normal animals studied.

The idea that the fat-body represents a general nutritive reserve to be utilized especially during hibernation and under conditions of unfavorable nutrition is perhaps the older point of view and has been held by Borradaille ('12), Holmes ('16), Milne-Edwards and von Wittich (Kennel, '12 b). Milne-Edwards also suggested the possibility of some connection of the fat-body with the reproductive activity. These investigators found support for their idea in the fact that the fat-body reached its maximum size in the fall and was reduced to a minimum at the close of hibernation—near the end of February or the beginning of March. Others, such as Gadow ('09) and Giglio-Tos (Kennel, '12 b), held that this fat reserve was used solely in the production of the sexual products, while Eckert and Gaupp (Kennel, '12 b) believed that it also functioned secondarily as a reserve organ for general bodily metabolism. Although Giglio-Tos found that maturation of the germ cells might take place in the absence of the fat-bodies, he emphasized the fact that conditions of nutrition must be exceptionally favorable for such maturation to take place. He also pointed out that the reduction of the fat-body to minimum size occurred seasonally at the end of the reproductive activity rather than after hibernation. The possibility that the function of the fat-body may differ with the species was strongly suggested by Meyer (Kennel, '12 b), who found that these organs had almost disappeared in one species of frog at the end of hibernation, whereas in another they did not tend to disappear until after the time of egg-laying.

Kennel ('11, '12 b), however, has studied the problem of the function of the fat-body in greatest detail. It is interesting to note that he considered the primary business of this organ that of a food reserve, both for the gonads in the sexual season and for the whole animal under unfavorable conditions of nutrition, such as occur during hibernation, while a secondary activity was that of a lymph gland. The utilization of the fat reserve by the gonads as well as the seasonal variation of these organs in both sexes is apparent from an examination of the graph (fig. 1) made from average weights of fat-bodies of frogs in eleven months of the year as given in his paper ('12 b). The curves indicate that the weight of the fat-bodies is at a maximum in October and November in both sexes, and at a minimum in April and May for the female and in March and April for the male, i.e., after utilization by the gonads. Moreover, marked development of the fat-bodies of the female as compared with those of the male appears to be correlated with the anabolic storage function of the ovary, in contrast to the catabolic function of the testis; and their greater reduction in the spring indicates their greater utilization in the formation of the eggs. Likewise, it is evident that the fat-bodies are used in general bodily metabolism because a decrease occurs during the winter months when, at least in the males, the fat reserve is not being wholly used by the reproductive organs. The second rôle, that of a lymph gland, was attributed to the fat-body mainly because during the spring and summer the infiltration of lymphocytes gave it a markedly lymphoid appearance.

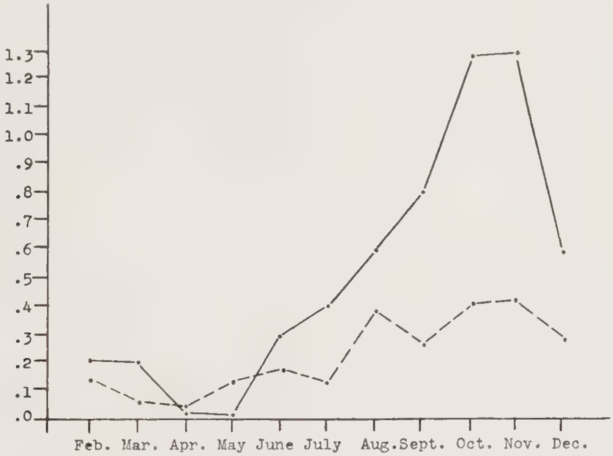

Fig. 1 Curves showing the weight of the fat-bodies of frogs in grams during eleven months of the year. Figures from Kennel ('12 b); — —, average weight of fat-bodies of males; ——, average weight of fat-bodies of females.

### *Removal of fat-bodies*

The first experimental attack on the problem consisted in the bilateral extirpation of these organs. Kennel ('12 a) removed both fat-bodies from frogs in November. Half of the animals lived until March, when they were killed. He claimed, as had Giglio-Tos earlier, that the removal did not affect the development of the sexual products and that the animals mated. Regeneration occurred in one animal. When the fat-bodies were removed in Triton, Aron ('24) found that there was an increased division of Sertoli cells and an increased discharge of the spermatozoa from the testes, provided there was no considerable stagnation of the efferent vessels to cause the cystic retention of the sperm and their subsequent phagocytosis. No record of unilateral removal of the fat-body was found.

### *Effect of inanition*

The results of starvation have been studied in Amphibia both from the point of view of loss in weight of body and organs and of the histological changes which occurred. In the present paper interest lies principally in the effects of inanition upon the fat-bodies and the gonads, and a search through the literature indicates that both of these organs undergo changes in starved animals. In the case of the former, a decrease in weight has been recorded, but no histological studies have been made; while in the latter, data have been secured by using both methods.

One of the most recent studies in which fat-bodies are considered is that by Ott ('24), who followed the changes in weight of the various organs of leopard frogs after variable periods of inanition, ten and one-half months being the longest starvation time for any group. The animals were killed when they had lost 10, 20, 30, 40, 50, and 60 per cent of their initial body weight. The relative loss of weight of the fat-bodies at all times during the experiment was greater than that of the body as a whole and occurred early, reaching a

77 per cent decrease in both sexes before there was any diminution in the body weight. Later, when the weight of the body as a whole had decreased 40 per cent, a loss of approximately 98 per cent was recorded in the fat-bodies of both sexes. Figure 2, constructed from Ott's data, shows the percentage decrease in the weight of the fat-bodies as compared with the body weight, and pictures especially their early diminution in size as the result of starvation. This reduction is in line with Morgulis's statement ('11) that in experimental inanition the greatest loss occurs during the first stages of inanition and diminishes subsequently. In this case it seems to point to a utilization of the reserve material for the maintenance of the normal condition of the body.

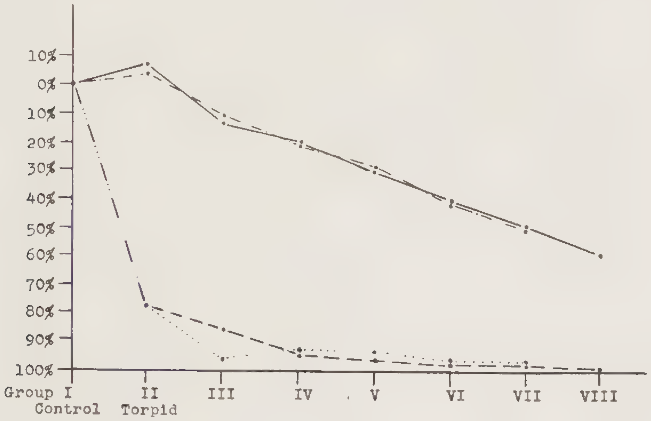

Fig. 2 Curves showing percentage change in the weight of the fat-bodies in leopard frogs compared with the percentage change in the weight of the body during inanition. Figures from Ott ('24); ——, percentage change in the body weight of males; —·—, percentage change in the body weight of females; —· · ·—, percentage change in the fat-body weight of males and females; — —, percentage change in the fat-body weight of males; . . . . . ., percentage change in the fat-body weight of females. Groups III, IV, V, VI, VII, and VIII are animals in which 10, 20, 30, 40, 50, and 60 per cent, respectively, loss in body weight had occurred.

Other studies agree with Ott's findings. After starving frogs for two months, Kennel ('12 b) recorded a decrease of 2 per cent of the body weight in females and 6 per cent in males, while the weight of the fat-bodies in females and males had been reduced 77 per cent and 72 per cent, respectively, as compared with the normal animals. Likewise, Heidkamp ('09) observed very lean fat-bodies in female tritons after starvation, and Swingle ('18) found that the large corpora adiposa of year-old tadpoles were so shriveled that they were almost unrecognizable after five months and ten days of being without food.

The effect of inanition upon the gonads takes a somewhat different course. In frogs, Ott ('24) found that, in contrast to the fat-bodies, the testes and ovaries did not undergo atrophy in early stages of the experiment, but only in later stages, after the body weight had markedly diminished. In males he recorded a loss of 89 per cent on the part of the testes when loss in body weight was 60 per cent, at which time the testes represented 0.037 per cent of the body weight as compared with 0.127 per cent in the normal controls. In females, when body weight had decreased 50 per cent, the ovaries had diminished to such an extent that they formed only 1.23 per cent of the body weight as compared with 13.8 per cent of that weight in the fall. One of the most drastic cuts in ovarian weight appeared to occur during the spawning season, even though the eggs were not laid. Before this season a loss of 6 per cent had occurred in ovaries of the group in which loss of body weight was 20 per cent, while, after it, ovarian loss reached 85 per cent, although reduction in body weight was only 30 per cent.

Likewise, the sensitivity of the gonads to starvation may be recognized histologically, as is clear from the papers of Heidkamp ('09) on female tritons, of Swingle ('18) on frog larvae, Champy ('21) on Triton alpestris, and Aron ('24) on urodeles. All of these workers find that the normal cycle of development is retarded, that inanition prevents sexual differentiation in immature animals and suppresses the maturation of the germ cells in adult individuals with subsequent resorption or degeneration of cells.

## MATERIAL AND METHODS

The animals selected for experimentation were adult aquatic specimens of Triturus (Diemyctylus) viridescens, the vermilion-spotted newt, which, according to Gage ('91), are two and one-half or three years old. They were collected during October, 1927, and throughout the experiment were kept in a room which was consistently cold. The experimental animals were divided into two divisions and handled according to the description in table 1.

TABLE 1

*Tabulation of experimental animals*

| GROUP | NUMBER | SEX | FED[1] OR UNFED | FAT-BODIES |
|---|---|---|---|---|
| Division 1. Animals in groups | | | | |
| I | 10 | Female | Fed | Both removed |
| II | 10 | Female | Fed | Right removed |
| III | 10 | Female | Fed | Left removed |
| Control [2] | 10 | Female | Fed | Normal |
| IV | 10 | Male | Fed | Both removed |
| V | 10 | Male | Fed | Right removed |
| VI | 10 | Male | Fed | Left removed |
| Control [2] | 10 | Male | Fed | Normal |
| VII | 10 | Female | Unfed | Both removed |
| VIII | 10 | Female | Unfed | Normal |
| IX | 10 | Male | Unfed | Both removed |
| X | 10 | Male | Unfed | Normal |
| Division 2. Animals in individual jars | | | | |
| A | 10 | Female | Fed | Both removed |
| B | 10 | Male | Fed | Both removed |
| C | 10 | Female | Fed | Right removed |
| D | 10 | Male | Fed | Right removed |
| E [3] | 5 | Female | Fed | Normal |
| F [3] | 5 | Male | Fed | Normal |
| Total, | 170 | | | |

[1] Food consisted of small pieces of earthworm or beef given at intervals of a day and a half during the experiment.

[2] Normal animals, not operated upon.

[3] Etherized and body cavity opened, but nothing removed.

Operations were performed under a binocular microscope. After etherization of the animal, a longitudinal cut was made in the body wall at the joining of the light- and dark-pigmented areas, halfway between the front and hind legs. Through this opening the fat-body was severed from its mesentery, removed, and the cut in the body wall was then sutured. If both fat-bodies were to be extirpated, this procedure was repeated on the other side; but in the individuals from which only one fat-body was taken the body wall was cut but the fat-body not removed on the second side. The animals serving as controls for the individuals and the unfed groups were etherized and cut on both sides so that the effect was the same, minus the actual removal of the fat-bodies (table 1). After the groups were complete, throughout the duration of the experiment only thirteen animals died —a mortality rate of 8.7 per cent, 69 per cent of which occurred in the unfed animals.

The animals in groups I to X were killed in the following sequence: three after 90 days, three after 120 days, and the remainder after 150 days. Half of each group of individuals (groups A to F) were killed after 90 days, and the other half, after 120 days. The operations were done between the last part of October and the middle of December, and the animals were killed from January through the first part of April. Gonads and fat-bodies were fixed in Zenker's fluid or picro-aceto-formol, and stained in Mayer's or Delafield's hematoxylin and eosin. Fixation of the fat-bodies with Benda's fluid was tried, but discarded as inferior to other reagents.

## OBSERVATIONS

All the animals were weighed soon after the beginning of the experiment and again when any of a group were killed. This series of weights was not continued over a long enough period of time to give any conclusive data as to the seasonal variation. An increase in the weight of all the fed animals indicated that captivity was not detrimental to their well-being, while unfed animals showed a gradual diminution in body weight. The removal of the fat-bodies caused no apparent difference in the general body weight of the animals of either the fed or the unfed groups.

TABLE 2

*Tabulation of cases of regeneration of fat-bodies in fed and unfed Triturus (Diemyctylus) viridescens*

| GROUP [1] | ANIMALS KILLED 90 DAYS AFTER OPERATION | | ANIMALS KILLED 120 DAYS AFTER OPERATION | | ANIMALS KILLED 150 DAYS AFTER OPERATION | | TOTAL | | PERCENTAGE OF REGENERATION WITHIN GROUPS |
|---|---|---|---|---|---|---|---|---|---|
| | Chances for regeneration | Actual regeneration | Chances for regeneration | Actual regeneration | Chances for regeneration | Actual regeneration | Chances for regeneration | Actual regeneration | |
| I ♀f2 | 6 | 4 | 6 | 2 | 6 | 2 | 18 | 8 | 44 |
| II ♀f1 | 3 | 1 | 3 | 2 | 4 | 2 | 10 | 5 | 50 |
| III ♀f1 | 3 | 2 | 3 | 3 | 3 | 2 | 9 | 7 | 78 |
| IV ♂f2 | 6 | 2 | 6 | 5 | 8 | 3 | 20 | 10 | 50 |
| V ♂f1 | 3 | 1 | 3 | 1 | 5 | 4 | 11 | 6 | 55 |
| VI ♂f1 | 3 | 0 | 3 | 2 | 4 | 3 | 10 | 5 | 50 |
| VII ♀u2 | 6 | 0 | 6 | 0 | 4 | 0 | 16 | 0 | 0 |
| IX ♂u2 | 6 | 0 | 6 | 0 | 4 | 0 | 16 | 0 | 0 |
| A ♀f2 | 10 | 3 | 8 | 3 | | | 18 | 6 | 33 |
| B ♂f2 | 10 | 3 | 8 | 0 | | | 18 | 3 | 17 |
| C ♀f1 | 5 | 1 | 4 | 1 | | | 9 | 2 | 22 |
| D ♂f1 | 5 | 3 | 4 | 2 | | | 9 | 5 | 56 |
| Total, | 66 | 20 | 60 | 21 | 38 | 16 | 164 | 57 | |
| Percentage of regeneration, | 30 | | 35 | | 42 | | *35* | | |
| Total, omitting VII and IX, | 54 | 20 | 48 | 21 | 30 | 16 | 132 | 57 | |
| Percentage of regeneration in fed animals, | 37 | | 44 | | 53 | | *43* | | |

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[1] In this column, f = fed, u = unfed, 1 = one fat-body removed, 2 = two fat-bodies removed.

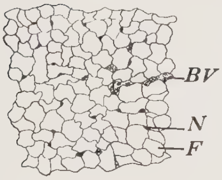
Regeneration of the fat-body occurred in 35 per cent of all the animals following either unilateral or bilateral removal (table 2). In estimating this percentage, bilaterally operated animals were considered to offer two chances for regeneration and unilaterally operated, one. No fat-bodies were regenerated in the animals of the unfed groups. Since inanition was not conducive to the regeneration of an apparently nutritive organ, possibly a fairer calculation of the percentage of regeneration would be one based wholly on the animals that were fed, which gives an estimate of 43 per cent regeneration as contrasted with the former 35 per cent (table 2). There was no striking difference in the regeneration of the fat-bodies in males and females. Whether the fat-body present represented regenerated tissue or an unremoved remnant was judged largely by its position and appearance. In a few cases the location of the fat at either end of the normal position of the fat-body and its color suggested that it was a part of the original organ and, therefore, these specimens were omitted from the calculation. In the other cases both the color, pale yellow or even white, and the position beside the testis in the normal mesentery indicated a probable regeneration. Histological examination revealed normal structure in the regenerated part. The average amount of the fat-body which regenerated, as indicated by gross measurement, was 11 per cent of the average size of the fat-body in the normal controls.

In the animals which had been fed, compensatory hypertrophy of the fat-body due to enlargement of the individual cells occurred following unilateral extirpation. Definite data on this point were available in the individuals with one fat-body removed. To determine whether there had been hypertrophy in these cases, the gross surface measurement of both fat-bodies was taken at the time of operation, the extirpated fat-body was fixed and studied histologically, and, when the animal was killed, the remaining fat-body was treated in the same way. Both the gross measurements and the histological structure of these were compared with those of normal individuals killed at the same time of year and of which parallel records had been taken. Although the surface measurement was not as accurate as the volume measurement would have been, it was thought indicative of significant changes because the shape of the body is band-like. In table 3 are recorded the data secured in this way. The percentage change clearly shows compensatory hypertrophy in both sexes, as does also a comparison of figures 3, 4, and 5, which represent a fat-

TABLE 3

*Tabulation of compensatory hypertrophy of fat-body after unilateral removal in fed Triturus (Diemyctylus) viridescens*

| GROUP | NUMBER OF ANIMALS | AVERAGE APPROXIMATE SURFACE AREA IN SQUARE CENTIMETERS AT OPERATION | | AVERAGE APPROXIMATE SURFACE AREA IN SQUARE CENTIMETERS 90 DAYS AFTER OPERATION | | AVERAGE APPROXIMATE SURFACE AREA IN SQUARE CENTIMETERS 120 DAYS AFTER OPERATION | | PERCENTAGE CHANGE |
|---|---|---|---|---|---|---|---|---|
| | | Right | Left | Right | Left | Right | Left | |
| C ♀ | 5 | .42 | .40 | | .50 | | | +25 |
| Right fat-body removed | 4 | .35 | .32 | | | | .33 | + 3 |
| E ♀ | 2 | .19 | .19 | .17 | .16 | | | —13 |
| Normal controls | 2 | .20 | .20 | | | .07 | .07 | —65 |
| D ♂ | 5 | .34 | .35 | | .47 | | | +34 |
| Right fat-body removed | 4 | .34 | .37 | | | | .54 | +46 |
| F ♂ | 2 | .17 | .18 | .14 | .14 | | | —20 |
| Normal controls | 3 | .24 | .27 | | | .20 | .19 | —24 |



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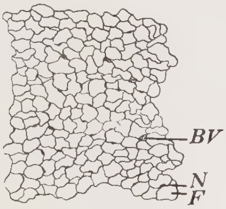

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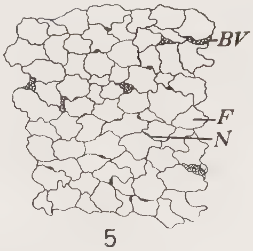

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Fig. 3 A portion of a section of fat-body of a normal control fed animal (F 1) killed in March. × 40.

Fig. 4 A portion of a section of fat-body removed from an animal (D 4) in December. × 40.

Fig. 5 A portion of a section of a fat-body showing compensatory hypertrophy. The fat-body of figure 4 was removed from this animal in December. The animal (D 4) was killed ninety days later, in March, and the remaining fat-body showed the hypertrophy pictured here. × 40. *BV*, blood vessel; *N*, nucleus of fat-cell; *F*, cavity in cell left after fat was dissolved out.

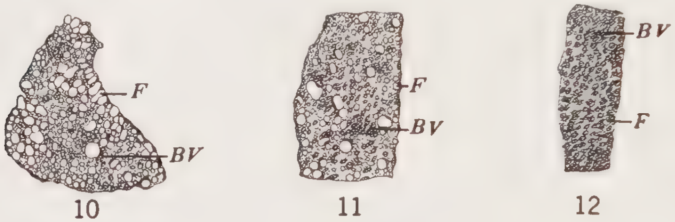
body at the time of operation, one 90 days after the removal of the first, and one from a normal animal at the time of the removal of the second fat-body. As may be seen in the table, after the removal of one fat-body in females the remaining one showed a 25 per cent increase after 90 days and a 3 per cent increase after 120 days, whereas the fat-bodies of the normal controls decreased 13 per cent in 90 days and 65 per cent in 120 days. Similarly, in the males the compensatory hypertrophy of this unremoved fat-body amounted to 34 per cent after 90 days and 46 per cent after 120 days, as compared with a loss in the controls of 20 and 24 per cent after 90 and 120 days, respectively. The smaller percentage increase of the remaining fat-body in the females after 120 days, together with the marked diminution in the fat-bodies in the control females of the same age, occurred in animals that were killed in April, when the development of the sexual products was at its height, and seems to point directly to a usage of the fat reserve by the gonads. These data on compensatory hypertrophy are in line with many studies on this reaction in other forms where one of a pair of organs is removed —such as kidney and lung of mammals, pronephros of Amphibia, etc.—and seem here, as in other cases, to indicate a response to a physiological deficit.

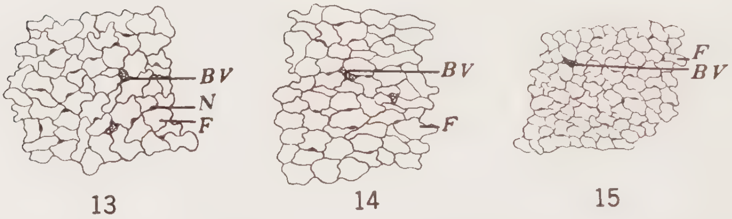
The effect on the gonads of the removal of both fat-bodies as shown by groups IV (fed males with both fat-bodies removed) and I (fed females with both fat-bodies removed) was a suppression of the development of the sexual products and subsequent degeneration, which appeared in progressive stages in animals killed after 90, 120, and 150 days. In the males the inhibition of development was evident in the relatively greater number of primary spermatogonia present in the testes as compared with the number in the testes of normal control animals killed at the same time (compare Humphrey, '21, p. 255). The cysts containing adult spermatozoa grew fewer in number and the majority of such cysts became heavily infiltrated with stroma cells and lymphocytes. Likewise, the secondary spermatogonia gradually lost their cyst formation and acquired pycnotic nuclei (compare figs. 6 and 7 of the testes of an operated and control animal).

As with the male, so the female reproductive organ ceased its activity after extirpation of the fat-bodies. The ovaries after 150 days (fig. 8) on comparison with those of the normal controls (fig. 9) showed a relatively greater number of small eggs, due partly to the cessation of their development caused by the removal of the fat-bodies and partly to the degeneration of the large and medium-sized ova. The degeneration appeared to be fatty in nature, occasionally associated with cellular infiltration. The débris of the larger eggs was gradually absorbed, and the end result was a small ovary. It was interesting to note in animals which had had only one fat-body removed that the ovary on that side was much smaller than the one on the normal side.

The effect of inanition on the gonads and the fat-bodies of normal animals was shown by groups X (normal males, unfed) and VIII (normal females, unfed). The fat in the fat-bodies gradually disappeared, leaving a solid mass of cells (figs. 10, 11, and 12) instead of the lacy network of the normal organ (figs. 13, 14, and 15). The surface measurements of such fat-bodies as compared with those of normal fed controls showed an average decrease of 90 per cent—which, of course, means a much larger decrease in volume. In the testes (fig. 16) the primary spermatogonia were not in evidence in great numbers. The secondary spermatogonia were semipycnotic, but their degeneration was not marked. The outlines of the spermatozoa became hazy, and the cyst boundaries were less distinct in this region because of the increased number of cells between the cysts. In the ovaries (fig. 17) degeneration was delayed and the eggs remained intact for a time while the fat reserve was being used, so that at the end of 120 days the decrease in the size of the ovary was less in this than in the other experimental groups. i.e., those with the fat-bodies out, both fed and unfed. No animals of this group lived for 150 days.

The combined effect of the removal of the fat-bodies and inanition proved to be the most detrimental treatment for the gonads, as seen in groups IX (unfed males with both fat-bodies removed) and VII (unfed females with both fat-bodies removed). In the testes (fig. 18) the secondary spermatogonia grew very pycnotic and shreddy, and fibrous tissue and infiltrating cells appeared among them. The spermatozoa were likewise scattered, with large regions showing cellular infiltration and fatty degeneration. The large and medium-sized eggs in the ovaries (fig. 19) in this group progressively and rapidly degenerated, and the end result was a very small ovary containing only small ova.





Figs. 10, 11, and 12 Portions of sections of the fat-bodies of unfed normal animals (X 1, 6, and 9) which had been starved 90, 120, and 150 days, respectively. × 40.

Figs. 13, 14, and 15 Portions of sections of the fat-bodies of fed normal control animals (C♂ 3, 6, and 10) after 90, 120, and 150 days, respectively. × 40. *BV*, blood vessel; *N*, nucleus of fat-cell; *F*, cavity in cell left after fat was dissolved out.

## DISCUSSION

The effect on the gonads of inanition and of removing the fat-bodies in Triturus may be summed up as a nutritional one. The results in both cases were, first, a cessation of development and, secondly, a degeneration of the genital products. As Ott ('24) observed in frogs, so here under conditions of inanition the gonads did not atrophy immediately, but continued to grow normally, for a time, at the expense of the fat-bodies so that the degeneration at a given time was less for these animals than for the others. When, however, this reserve was cut down and gradually exhausted, resulting in a fat-body resembling lymphoid tissue (compare Kennel, '12 a), development of the sex cells ceased and retrogression began (as had been observed by Heidkamp ('09), Swingle ('18), Champy ('21), Aron ('24), and Ott ('24)). Contrary to the findings of Kennel ('12 a) and Giglio-Tos in frogs, the removal of the fat-bodies in these newts resulted in almost immediate inhibition of development of the sexual products and even in their degeneration, although the animals were fed. The combination of the removal of the fat-bodies and inanition produced similar but speedier and more extensive results.

The degeneration which occurred in the gonads due to the lack of nutriment proceeded in a definite order. In the testes it started with the spermatozoa and continued to the secondary spermatogonia, the most differentiated cells being attacked first. This sequence is similar to that found by Siperstein ('21) in the testes of starved albino rats, by Loisel ('01) in those of a starved dog, and by Lindsay and Medes ('26) in those of guinea-pigs suffering from scurvy and inanition. The latter also reported the absence of spermatozoa. The present data would indicate that they had degenerated and that the débris had been resorbed. Champy ('21) found only indifferent cells and spermatogonia in the testes of Triton alpestris that had been starved, and it seems probable that this would have been the ultimate result in the present experiment if the animals had been kept for a longer time.

It was interesting to compare these data with those of Young ('27) as to the effects of heat on the testes of guinea-pigs. He claimed that degeneration of testicular cells occurred depending upon the state of activity of the cells at the time of the experiment, the order being: large primary spermatocytes, secondary spermatocytes, young spermatids, a few spermatogonia, and then older spermatids, spermatozoa in the seminiferous tubules, early primary spermatocytes, and, finally, the spermatogonia that were the most resistant. He asserted that a source of error might exist in the findings of investigators who held that degeneration attacked the most-developed cells first (thus reversing the order of development), unless the whole series were present, because the first stages of degeneration might not be observed. In the present experiment, however, the 90-, 120-, and 150-day animals formed a series with which the normal controls of the same number of days were compared. The fact that the animals were killed in the spring accounted for the absence of spermatids and spermatocytes, which are not normally present at that time, and their absence was not due to an earlier degeneration of these stages.

The eggs in the ovaries also regressed in a sequence similar to the developing sperm cells, the largest ones first, and so on according to size down to the small ones, as Heidkamp ('09) had observed in the female Triton. The degeneration and absorption of the large eggs naturally resulted in a marked decrease in the size of the ovaries. Whether or not the ovaries were used for the nourishment of other tissues under conditions of inanition, as Morgulis ('11) found in Diemyctylus and as Ott ('24) intimated for the frog, was not determined in this study. There was, however, abundant evidence that the fat-bodies contributed to the maintenance of the ovaries.

The data presented in this paper point to the function of the fat-body as a nutritional reserve for the use of the gonads. Both the lack of regeneration and the decrease in the size of the fat-bodies in unfed animals indicate that the reserve can be stored and maintained only under favorable conditions of nutriment. Evidence for the dependence of gonads on fat-bodies is clear in the observations on normal animals (both sexes) and those with one fat-body removed. In April, when the eggs were largest, the fat-bodies of the normal female controls had suffered a loss of 65 per cent, as compared with a loss of 13 per cent in those killed one month earlier (table 3). The 3 per cent hypertrophy in the females killed in April, as compared with the 25 per cent of those killed in March, suggested that all fat-bodies had undergone a similar hypertrophy, but that the April ones had utilized some of the reserve. The extensive development of the ovaries of these individuals was especially conducive to this conclusion. Although of lesser degree than in the female, the decrease of the fat-bodies in the male controls also points to the gonad-fat-body dependence, and in both sexes the data fall in line with those of Kennel ('12 b) from which the graph in figure 1 was made. The utilization of the fat in the fat-bodies for the maintenance of the testes and ovaries in normal state during the first stages of inanition presents a further bit of evidence as to their rôle. That the fat-bodies are not able to sustain the gonads over a long period of time, as they normally seem to during hibernation, is probably due to the fact that the more active conditions of the animal in the laboratory do not induce the lower metabolic rate which exists during hibernation.

The evidence for the use of the fat-body in general body nutrition, especially under unfavorable conditions, was not investigated in this study. However, it should be pointed out that the unfed groups with fat-bodies fared no better than those without fat-bodies, as far as the general body weight was concerned.

## SUMMARY

1. The effect of inanition on the fat-bodies was a utilization of the fat for the normal maintenance of the gonads.

2. The effect both of the removal of the fat-bodies and of inanition on the gonads was a nutritive one. Both resulted in the cessation of the development of the sexual products, which was followed by a degeneration of those products already formed, the most advanced stages of development going first.

3. Removal of the fat-body was followed by regeneration in 35 per cent of the cases.

4. Compensatory hypertrophy of the remaining fat-body followed unilateral removal.

5. The results of the experiment led to the conclusion that the function of the fat-body was a nutritive one, connected with the development of the sexual products, and especially essential in the development of the eggs of the female.

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## PLATE 1

### EXPLANATION OF FIGURES

6 Section of the testis of a normal fed control animal (C♂ 8), 150 days after the beginning of the experiment. × 15.

7 Section of the testis of a fed animal (IV 9) from which both fat-bodies had been removed 150 days previous to the time the animal was killed. × 15.

8 Section of the ovary of a fed animal (I 9) from which the two fat-bodies had been removed 150 days previous to this. × 7½.

9 Section of the ovary of a normal fed animal (C♀ 4), 150 days after the beginning of the experiment. × 7½.

16 Section of the testis of a normal animal (X 9) which had not been fed for 150 days. × 15.

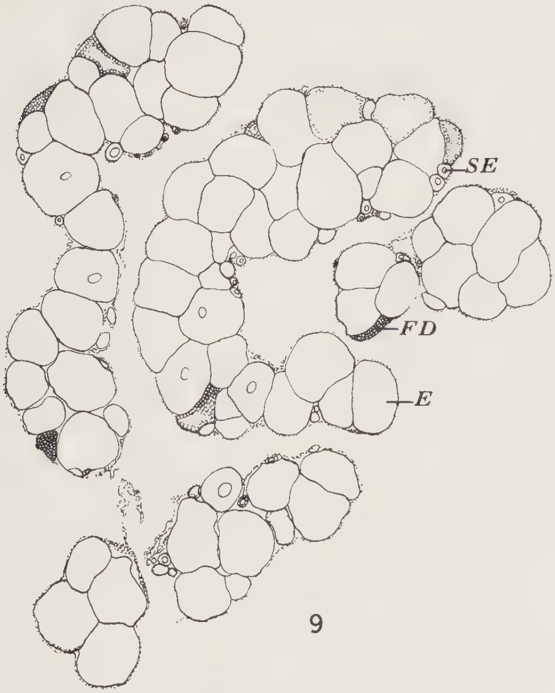
17 Section of the ovary of a normal animal (VIII 4) which had not been fed for 150 days. × 7½.

18 Section of the testis of an animal (IX 7) from which both the fat-bodies had been removed 150 days before and which had not been fed during that time. × 15.

19 Section of the ovary of an animal (VII 7) from which the fat-bodies had been removed 150 days before and which had not been fed during that time. × 7½.

### ABBREVIATIONS

*P SPG*, primary spermatogonia
*S SPG*, secondary spermatogonia
*SPZ*, spermatozoa
*D SPG*, actual degenerative region of secondary spermatogonia
*E*, egg
*SE*, small egg
*FD*, brown, fatty degeneration



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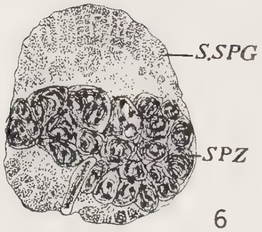

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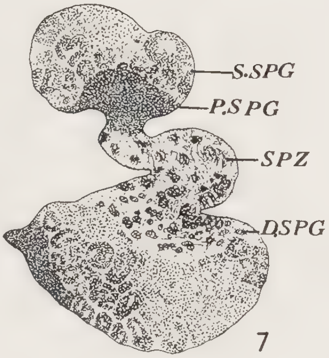
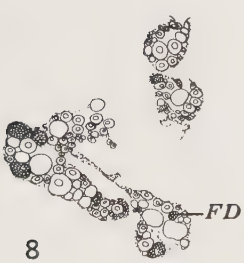

S.SPG
P.SPG
SPZ
D.SPG

7



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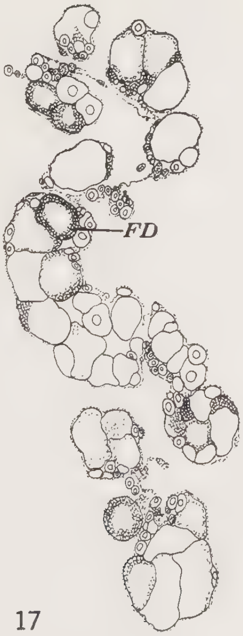

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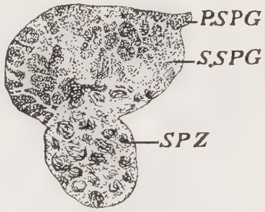
P.SPG
S.SPG
SPZ

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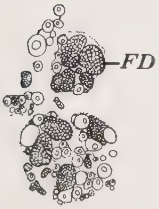

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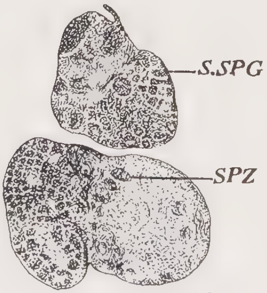

18

# MERCUROCHROME (DI-BROM OXY MERCURI FLUORESCIN) AS A FUNGICIDAL AGENT IN THE GROWTH OF AMPHIBIAN EMBRYOS

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In the present communication we wish to report some observations which we have made upon the application of Mercurochrome as a fungicidal agent in the growth of Amblystoma embryos. During the operating season of 1928 (March to May) the conditions under which we worked favored the growth of molds, and the larvae which had been employed in various types of tissue-grafting experiments early became infected.

The chief mold which we encountered was Saprolegnia, although there seemed to be various other strains which we, unfortunately, did not identify. The first infections occurred in March. Several series of larvae which had been operated upon early in March and which were in healthy condition several weeks after the operation suddenly began to show the presence of mold. The growth occurred usually in the form of small tufts which grew out from the gills, the legs, and from the sides of the body, particularly along the lateral-line sense organs. In a number of cases the infection was so severe that death ensued before treatment could be applied.

Attempts were made in the beginning to reduce the infection by changing the animals daily into fresh spring water which had previously been aerated. This did not reduce the infection. At the suggestion of Doctor Noble, of the American Museum of Natural History, we then tried to reduce the growth by placing copper coins in the water—a method which he employed with partial success in treating older animals.

This also seemed to have no effect. We then learned from Mr. Otto Gneiding, a breeder of fancy fish in Ridgefield Park, New Jersey, that he was able to prevent the growth of molds which frequently develop in the wounds of fish by adding Mercurochrome to their aquaria. With this information at hand, we set out to investigate the possibilities of using this antiseptic in the case of infected embryos. As a preliminary test, several animals with well-developed tufts of mold on the fore limbs and gills were placed in a solution of 1:500,000 Mercurochrome made up in spring water.[1] In twenty-four hours after treatment, the hitherto vigorous hyphae had collapsed, and the whole growth was easily removed with fine forceps. No effects upon the larvae were noticed. After the mold had been removed, the larvae were placed back in spring water. In several other cases, however, in which the mold had already covered the whole body, Mercurochrome treatment had no effect, and the animals died.

Investigations were then made in an attempt to find a concentration which would destroy the mold and in which larvae could be reared without injury and retardation of growth. This was of utmost importance, since temporary treatment, although effective, would be of little value if the animals had to be placed back in spring water where reinfection took place so readily. At this juncture we were encountering a general growth of mold upon many of the larvae in a number of experimental series. A concentration of 1:750,000 was made up, and all infected animals of the various series were placed in this solution. The larvae at this period were entering the feeding stage (two to three weeks after tail-bud stage). The results of the treatment are shown in table 1. In this table four series are listed. There were sixty-nine animals, of which fifty-six showed the growth of mold to varying degrees.

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[1] Tap-water could not be used in the experiments, because it was found to be toxic. Embryos placed in tap-water when in stage 29 (tail-bud stage) showed no effects as regards growth and reactions up until the time when the circulation began. The larvae then ceased to grow, the gill filaments necrosed, epithelial sloughing occurred, the circulation failed, and death ensued. Control embryos in aerated spring water underwent normal development.

In twenty-four to forty-eight hours after treatment, the mold was either completely sloughed off or was in the process of sloughing.

As a control to these experiments we had a number of cases in which molded animals were placed in fresh spring water. The mold did not disappear until after these animals were given treatment.

The treated animals were kept in the Mercurochrome solution up until the time they were fixed, which ranged from fifteen to fifty days. It was found that after several days the solution began to lose color. A fresh solution of 1: 750,000 concentration possesses an iridescence and has the color of weak eosin. After several days the solution begins to deteriorate. This deterioration appears to be hastened by the presence of dead organic matter in the aquaria which accumulates from various forms of unconsumed food. For this reason, the solution was entirely changed from time to time, or enough of the new solution was added to the aquaria to bring the color approximately up to that of a 1: 1,000,000 concentration. In several series of experiments (series CCRU), in which the mold had developed to only a slight degree, a concentration of 1: 1,000,000 was employed and appeared to inhibit further growth.

TABLE 1[1]

*Showing effects of Mercurochrome treatment in various series of experimental embryos which developed molds*

| SERIES | NUMBER OF ANIMALS | NUMBER OF ANIMALS INFECTED | AVERAGE AGE OF LARVAE WHEN INFECTED | CONCENTRATION OF MERCUROCHROME | RESULTS |
|---|---|---|---|---|---|
| AA2S | 19 | 17 | 2–3 weeks | 1: 750,000 | Mold disappeared in all cases treated in 24–48 hours, depending upon severity of infection. All larvae survived. |
| AS4 | 21 | 18 | 2–3 weeks | 1: 750,000 | |
| LETH | 20 | 13 | 2–3 weeks | 1: 750,000 | |
| HS4 | 9 | 8 | 2–3 weeks | 1: 750,000 | |

[1] Animals badly infected were placed in a concentration of 1: 500,000 for twenty-four to forty-eight hours, after which the solution was diluted to 1: 750,000 or 1: 1,000,000.

Many animals which were reared for several weeks in the Mercurochrome and which showed no evidence of infection were allowed to remain in the solution without further change. Although deterioration took place in the course of several weeks, most of these cases remained free of mold. In cases of reinfection, a new solution was employed.

No deleterious effects of the Mercurochrome upon the experimental larvae were observed. The feeding reactions were normal and the growth rate was in no way impaired. Varying the concentrations within the limits of 1:500,000 to 1:1,000,000 appeared to have no effect. In fact, several cases are recorded in which older unoperated larvae, which became infected about forty-five days after tail-bud stage and which were treated with a concentration of 1:210,000, suffered no deleterious effects of this strong solution.

The application of Mercurochrome treatment to experimental animals usually did not begin until from two to three weeks after the tail-bud stage. At this time the larvae are entering the feeding stage and conditions enhancing mold growth are then introduced, because food is placed in the aquaria, thus exposing the dish more frequently to spores. Much of this food is not consumed, and with the accumulation of dead organic matter the mold growth usually increases rapidly.

During the early part of the experimental season we were very successful in curing infected animals and inhibiting further growth in the aquaria. Later in the season, however, we encountered a more vigorous or resistant mold which grew heavily upon the dead daphnia in the aquaria, even in the presence of stronger concentrations of Mercurochrome solutions (1:250,000) than previously employed. The fact that under these circumstances only a few animals became infected was regarded by us as indicating that the Mercurochrome was active in inhibiting growth upon the living larvae. This was suggested further by the fact that larvae which died from other unknown causes developed mold very rapidly, as did the dead daphnia, but mold which had gained a foot-hold on healthy living larvae nearly always sloughed off in twenty-four hours in the presence of the Mercurochrome solutions employed.

Although concentrations of Mercurochrome ranging in strength from 1:1,000,000 to 1:250,000 showed no deleterious effects upon larvae which were several weeks old when first treated, a series of observations upon early embryos did show marked effects of the Mercurochrome. For these observations, a series of normal embryos between stages 29 and 37 were placed in concentrations of Mercurochrome varying from 1:100,000 to 1:1,000,000. These solutions were changed every second day. The results are given in tables 2 and 3. From table 2 it is seen that embryos survived progressively longer and longer periods as weaker concentrations were employed. Embryos in a 1:100,000 concentration survived only one day, whereas those in a 1:1,000,000 survived as long as thirty days. During the periods of treatment growth was greatly retarded and the larvae early developed abnormal postures and reactions. These developed earlier and were most marked in the stronger concentrations (table 3). The abnormalities consisted, in all cases, of kyphosis which was accompanied by a failure of progressive swimming. In many cases the animals, when stimulated, underwent vigorous tremors. The specific lethal effects of the Mercuro-

TABLE 2

*Showing survival (in days) of normal Amblystoma embryos (stages 29–37) grown in different concentrations of Mercurochrome*

| CONCENTRATION | NUMBER OF ANIMALS EMPLOYED | DAYS SURVIVING | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 3 | 6 | 8 | 9 | 10 | 13 | 15 | 18 | 21 | 24 | 27 | 28 | 30 |
| 1:100,000 | 10 | 10 | | | | | | | | | | | | | |
| 1:150,000 | 16 | | 2 | 9 | 5 | | | | | | | | | | |
| 1:250,000 | 11 | | | 1 | 2 | 1 | | 7 | | | | | | | |
| 1:500,000 | 11 | | | | | | | 1 | 10 | | | | | | |
| 1:750,000 | 9 | | | | | | 1 | | 2 | 1 | 5 | | | | |
| 1:1.000,000 | 7 | | | | | | | | | | 1 | 1 | 1 | 1 | 3 |

chrome upon these early larvae have not been determined. Whatever they are, they have no effect upon larvae several weeks of age, for these older larvae can be grown continuously in solutions as strong as 1:500,000 without any noticeable interference with normal growth and reactions.

TABLE 3

*Showing time (in days) when normal Amblystoma embryos (stage 37) treated with Mercurochrome first exhibited abnormal reactions*

| CONCENTRATION | DAYS |
|---|---|
| 1:150,000 | 2 |
| 1:250,000 | 6 |
| 1:500,000 | 8 |
| 1:750,000 | 8 |
| 1:1,000,000 | 8 |

## SUMMARY

1. Mercurochrome in concentrations ranging from 1:500,000 to 1:1,000,000 has been employed successfully as a fungicidal agent in the growth of Amblystoma embryos.

2. Larvae several weeks of age can be successfully treated without any deleterious influence upon the animal. Continuous treatment over a period of two months has no noticeable effect upon growth. Animals feed and react normally in the presence of the fungicidal solution.

3. Molds growing on larvae usually slough off within two days when in the presence of Mercurochrome (concentration 1:750,000).

4. Molds of resistant strains grow upon dead organic matter (unconsumed food) in the presence of Mercurochrome (concentration 1:500,000), but this concentration usually inhibits growth upon healthy larvae.

5. Fresh concentrations of 1:500,000 and 1:750,000 possess an iridescence and have the color of weak eosin. This color fades within a week and the solution loses its effectiveness. Its deterioration appears to be hastened by the presence of dead organic matter.

6. Early embryos (stages 29 to 37) subjected to continuous treatment of Mercurochrome in concentrations ranging from 1:1,000,000 to 1:100,000 develop abnormal reactions, and eventually die. Embryos survive progressively longer and longer as weaker concentrations are employed. Only young embryos are lethally affected.

# THE FREEMARTIN CONDITION IN SWINE

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EIGHT PLATES (THIRTY-TWO FIGURES)

## INTRODUCTION

In a previous paper (Hughes, '27) a report was given of the freemartin situation occurring in swine. Forms of embryonic sex intergrades were found existing under the same conditions that, in cattle, invariably lead to the production of the sterile freemartin. In both species the establishment of a common fetal circulation between an opposite-sexed pair of individuals gives rise to profound modifications in the genital system of the female cotwin, in which normal female growth is more or less completely checked and male growth unduly stimulated.

The extension of this condition to another species serves somewhat to strengthen the hormone theory of Lillie ('16) and of Keller and Tandler ('16)—a theory first promoted as an explanation of this type of intersexuality in cattle. The conclusion reached by these authors was that the freemartin is a zygotic female whose sex has to a certain extent been reversed under the stimulus of male sex hormones circulating in the fetal blood stream and passed into her system through vascular intercommunications. This interpretation leads to further conclusions regarding the actual mechanism of sex differentiation in mammals, in that it necessarily involves the assumption of specific sex hormones as the stimulus controlling the process of sexual differentiation in the embryo. These hormones, supposedly arising in the gonads of the embryo, may, on account of their specific nature, set up considerable disturbance when introduced into an individual of the opposite sex, in the case of the cattle freemartin sometimes going so far as to change a normal zygotic female into an individual with more or less complete internal structural organization of a male.

The fact that, in the case of the cattle, this transformation takes place in the female only, without there being any interference of normal development in the male cotwin, is suggested as being due to the precocious development of male hormones (Lillie, '17). Thus the early sex differentiation of the female presumably takes place solely under zygotic control, while that of the male is early subject to hormone stimulus. Hence, upon introduction of this male stimulus into the female through the vascular anastomoses, all further construction of her sex organization is under its direct control, and a process of sex reversal sets in.

## CHORIONIC FUSIONS IN THE PIG

Although the freemartin theory permits general applicability among mammals, at the present time the condition is known to occur only in three species: cattle, the goat (Keller, '20), and the pig. Intergrades in various other species have at times been attributed to the same cause, but in that they lack the crucial evidence of placental anastomoses, conclusions regarding their origin are not decisive. The condition essential for the production of the freemartin intergrade is the establishment of a common circulation between an opposite-sexed pair of individuals, the vascular connections following fusion of originally separate blastocysts. The probability of fulfillment of this condition is practically limited to species with the diffuse type of placenta; hence we would expect the pig, especially in view of the crowded condition of its uterus, to offer exceptional opportunities for the study of the freemartin situation. As a matter of fact, if the placenta of the pig were, strictly speaking, of the 'diffuse' type, this might be true, and chorionic fusions would then be the rule rather than the exception. But such a condition could not possibly exist, as it would mean the absence of fertile females; and therefore we find that the placenta of the pig and of all multiparous animals is especially adapted to make the fusion of chorions under normal conditions an improbability. In the pig this seems to be effected by means of the non-placental apices of the chorion. We find that these terminal regions remain thin and devoid of blood vessels, so that at the time when the embryonic membranes are most susceptible to fusions, probably during the process of vascularization, they prevent the vascular, and therefore more sensitive, portions of adjacent vesicles from coming into direct contact with one another. Similar regions, the so-called 'diverticula allantoidis' of the early embryologists, are found in sheep, but are very poorly represented in cattle.

That these terminal areas in the pig are effective in preventing fusions is demonstrated by the fact that end-to-end fusions are extremely rare, while side-by-side fusions occur relatively frequently. Blastocysts which because of faulty spacing come to lie side by side, fuse for the whole length, as here the placental walls of the two vesicles come into direct contact. In either case, end-to-end or side-by-side, obliteration of the fused chorionic walls takes place and results in a single chorionic cavity occupied by two embryos, each with its amnion, allantois, and yolk sac.

Fusions resulting in the monochorionic condition evidently take place only early during embryonic life, for during the latter half of pregnancy there is extensive overlapping, telescoping, and adhering of adjacent vesicles without establishment of the monochorionic twinning condition.

The distribution of fetal blood vessels in these cases of twinning is always irregular. The vessels of one twin are not confined to its own allantoic walls, but wander without discrimination into whatever portion of the placenta is available, and consequently provide excellent opportunities for vascular anastomoses between the two individual systems.

## MATERIAL AND METHODS

The cases described in my previous paper were in a fairly early stage of development, sizes of the embryos ranging from 6 cm. to 9 cm. At this stage sexual differentiation of the genital ducts has barely commenced, so the study of sexual modification was confined to the gonads. With the object of obtaining stages showing later differentiation, a further search was made for material, and four more cases were collected, all in later stages of development.

The material was collected on the hog-killing floor of the Swift & Co. local packing house. Selecting only uteri in the second half of pregnancy, it was necessary to examine very carefully from 400 to 500 uteri in order to find these cases and a few others in which the twins were either one-sexed or did not show the presence of vascular connections. Approximately, the ratio of monochorionic twinning is one to every thirty or forty uteri, or perhaps a little higher than this in the earlier stages of development, as the death rate is fairly high.

The presence of monochorionic twinning can sometimes, but not always, be detected before opening the uterus. Normally, the spacing in the uterus is regular and the embryonal swellings are separated from each other by uterine constrictions. Accordingly, where a couple of embryos are felt in close approximation or where the customary constriction is lacking, one may expect to find a pair of twins.

To demonstrate the presence of a common circulation, the fetal vessels were injected with colored-starch masses. Figure 1 shows a pair of twins with membranes cut to expose the blood vessels. The injection was made into the male (right) umbilical cord only, the veins being injected with a Prussian-blue mass and the arteries with yellow ochre. In the case illustrated, both arterial and venous connections are strong, and the coloring mass passed directly across and entered the female umbilical cord. This condition is rather unusual in the pig, for in most cases the connections are confined to the veins and the arterial anastomoses, if present at all, are very weak. In one case a large artery passing directly from one twin to the other was blocked and degenerating at one place in its course, and increased pressure on the injection mass merely produced rupture in the vessel. This blockage was probably the result of the collision of the blood streams approaching from opposite directions, and had the effect of redirecting the blood along other channels. In cattle the reverse condition prevails, in that the venous connections are rare and the arterial more frequent (Lillie, '17).

In each of the four cases the urogenital systems of both male and female embryos were removed and completely sectioned. Before entering into a detailed account of these cases it might be well, for purposes of comparison, to describe briefly the normal development of the sex organs in the pig.

## NORMAL DEVELOPMENT OF THE REPRODUCTIVE SYSTEM OF THE PIG

*2.5-cm. embryo.* The gonads at this stage can for the first time be identified as testes or ovaries. The ovaries are characterized by the proliferation of secondary sex cords (cortex) from the germinal epithelium, while in the testes the epithelium has ceased growth and is separated from the primary sex cords by a tunica albuginea (Allen, '03). At this stage the mesonephros is in an active condition and the mesonephric ducts are accordingly well-differentiated tubes (figs. 2 and 3). Müllerian ducts are in the process of formation and extend posteriorly in form of solid cords for about half the length of the mesonephros. In both sexes they open anteriorly into the coelom by means of the ostium.

*5-cm. embryo* (figs. 4 and 5). At this stage the müllerian ducts have reached the urogenital sinus and have united posteriorly in the region of the genital cord. In this region, the future region of the uterus and the uterus masculinus, the ducts are canalized, but they end blindly in the walls of the sinus in both sexes. In the male the remainder of the müllerian duct, with exception of a small portion in region of the ostium, normally remains solid and does not attain a lumen. In the female canalization is in progress, commencing in the region of the ostium and proceeding posteriorly.

*12-cm. embryo.* Shrinkage of the mesonephros is now in progress, and there is accordingly a decided narrowing of its duct in both sexes. The müllerian duct in the female has attained its lumen throughout, although it has not yet perforated the walls of the sinus. In the male degeneration of the müllerian duct is in progress, and its course has become interrupted in several places (figs. 6 and 7).

*15-cm. embryo.* Further differentiation of the genital ducts is now rapid. At this stage the definitive condition of the genital ducts and associated structures has practically been attained (figs. 8 and 9). In the male the müllerian ducts have disappeared with the exception of the vestigial uterus masculinus and ostium, while the mesonephric ducts are forming connections with the tubules of the testis; the rete tubules are commencing to attain lumina. The seminal vesicles are well advanced, and prostate and bulbo-urethral glands are also present. In the female the müllerian ducts are well differentiated and widen considerably in the region of the uterus. The posterior extremity is still solid, but the core of cells occluding its lumen is loosening up to form a continuous passage through to the vestibule. The mesonephric ducts are represented posteriorly by the ducts of Gärtner and anteriorly by the epoöphoron and the paroöphoron.

## DESCRIPTION OF CASES

*Case A.* This is the youngest of the four cases, the measurements of the embryos being: male, 11 cm., and female, 10 cm. The external genitalia of both appeared normal.

The gonads measured: male, 4 mm. × 2 mm., and female, 1.2 mm. × 0.5 mm. The small size of the gonads in the female is the first evidence of sexual modification and is very pronounced in all cases.

On sectioning, the reproductive system of the male appeared to be normal in every respect. The müllerian ducts were fragmentary solid cords and the mesonephric ducts and gonads were pursuing their regular course of development.

In the female the minute size of the gonads is seen to be due not only to the absence of the thick cortical zone which characterizes the normal ovary at this stage, but also to the reduction of the medullary region. Hence the gonad is reduced to a structure consisting chiefly of rete and an unorganized mass of compact connective tissue. Large blood vessels penetrate this mass, and there are also clumps of sex cells scattered in it. The cells in this case are difficult to identify as belonging to the medullary or cortical region. Those around the periphery are undoubtedly cortical cells, as they can be seen in places growing in from the germinal epithelium. The inner cells are probably the remains of the medullary cords, as some of them seem to be directly associated with the rete, and they are in places distinctly separated from the cortex by an albuginea. Strands of tissue radiate from the rete, dividing this inner mass into separate areas, representing what is perhaps an attempt at formation of seminiferous tubules (fig. 23). The cortical cells are very scanty in number, although they appear to be quite healthy. The rete deeply penetrates the gonad, and on leaving it becomes associated with several malpighian bodies. The reproductive ducts are male in character. Figure 14 represents a reconstruction of the system from serial sections. It is noted that the müllerian duct, which should in a normal female be a completely canalized tube, is represented by a faint solid cord which has disappeared in places. Modifications of the wolffian ducts could not be demonstrated, as these ducts are not sexually differentiated at this stage even in the normal. Therefore, it is not known what course they would follow.

*Case B.* Both male and female fetuses measured 15 cm. and in both the external genitalia appeared normal. In the male twin the testes were normal in size and appearance, but in the female the gonads were so minute that they could hardly be detected with the naked eye. They were, in fact, no wider than the accompanying genital ridges (figs. 13 and 13 a). The saccus vaginalis and gubernaculum were typically male in form.

Figure 17 represents the male system reconstructed from serial sections. The testes were normal in every way, and large seminal vesicles and other glands were also present. There was, however, one abnormality present, and that was in the condition of the müllerian duct on one side. As shown in the figure, this duct is represented for a considerable length of the genital ridge by a canalized tube comparable with the female oviduct. Although it has become intermittent, the portions present are wide in diameter. Its relative size is shown in figure 31, a photomicrograph of a cross-section through the genital ridge just posterior to the testis. This is the only case I have found in which there is any abnormality in the male system. That these ducts which normally do not attain lumina in the male should persist in this form is highly suggestive of female influence. But there is also the possibility the condition has no bearing on the subject at all, and that its appearance may be nothing more than a coincidence.

The structure of the genital system of the female is illustrated in figure 16. It is seen that for the greater part of the length both wolffian and müllerian ducts have completely disappeared. A section through the genital ridges at a level marked $x$ in figure 16 shows a solid mass of unorganized cells as opposed to the normal (compare figs. 29 and 32); and a section through the genital cord shows the absence of the uterus (compare figs. 27 and 28). The anterior tubules of the mesonephros are persisting in form of a reduced epididymis and are attached to the solid rete cords. The gonads, like those in the previous case, consist largely of rete and unorganized stroma. Figure 20, a photomicrograph of a section through the gonad, shows its relative size compared with a normal ovary (fig. 19). The structure of the gonad is better represented in figure 26. Occupying the center is the rete, which for the most part consists of solid cords of cells, but occasionally canalized tubules also. It is doubtful whether these hollow tubules represent any irregular growth of rete, as they may be occasionally observed in normal ovaries. The germinal epithelium in places has ceased growth and is reduced to a layer of flattened cells, but in other places its activity is indicated by the proliferation of cells into the stroma beneath. These cells, which represent the cortical portion of the ovary, are large and have an abundance of cytoplasm. They are much more abundant than in the gonads of case A. They lie scattered in clumps and do not appear to be undergoing degeneration. The medullary region is not so well defined, but is in form of a compact mass of unorganized cells in the region surrounding the rete.

*Case C.* Measurements of the male twin were 16 cm. and of the female, 14.5 cm. External genitalia were normal in both. The dissected urogenital system is shown in figures 12 and 12a, and here again the gonads are exceedingly minute. The saccus vaginalis and the gubernaculum are again male in structure.

This case repeats to a certain extent the previous, with the exception that the male twin was entirely normal and showed no signs of persistence of the müllerian ducts.

The size of the gonad of the female is illustrated in figure 22 and its structure, in figure 25. Here again the rete is the chief feature, occupying the central position and consisting of cords part solid and part with lumina. The germinal epithelium is partly reduced to a layer of flattened cells, but proliferation is also taking place, and a considerable number of sex cells are scattered in the cortical region. These cells are easily recognized by their size and their large clearly defined nucleus. Most of them appear to be healthy, but others are undoubtedly undergoing degeneration.

The medullary region is an unorganized mass of cells containing no trace of any definite primary sex cords.

The reproductive ducts resemble those of the previous case (figs. 15 and 30).

### *Withdrawal of male influence*

*Case D.* An additional case was found in which the male cotwin had died at the 12-cm. stage, while the living female measured 15 cm. The male was in a mummified condition and therefore useless except in so far as it showed the effect of the withdrawal of male stimulus. Vascular anastomoses were present in that portion of the placenta still occupied by the allantois of the male, but the greater part of its allantois and blood vessels had withdrawn from the placenta.

On dissection, the female gonads were seen to be considerably reduced in size, being about half the size of normal ovaries of the same age. Moreover, blood vessels could be seen coursing on the surface as on normal testes. The genital ducts, on sectioning, proved to be those of a normal female, nothing more than the customary vestiges of the wolffian ducts remaining. The small size of the gonads was seen to be due to the reduction of the cortical zone which normally forms the bulk of the gonad (compare figs. 19 and 21). The cortex occupies a narrow zone at the periphery, and in places is absent altogether, allowing the blood vessels in this region to appear at the surface. The cells of the cortex appear to be normal and in an active condition. The medullary region is relatively large and contains numerous clumps of sex cells which show no signs of degeneration.

From this case one might conclude that the withdrawal of male stimulus permits the return of full normal female growth. The fact that withdrawal was effected at the critical moment for sexual differentiation of the genital ducts, i.e., 12 cm., explains their female character, for if the withdrawal had been at a slightly later date, undoubtedly modification would have progressed too far to permit full response to the new female stimulus. It is interesting, in view of the discussion to follow shortly, to note that the presence of a comparatively well-differentiated medullary region in the gonads has no effect on sex differentiation of the ducts. It cannot, therefore, itself be producing any male hormone. No interstitial cells could be recognized in any of the four cases described herein.

## DISCUSSION

The material just reviewed shows that the intermingling of male and female blood during early embryonic development has a profound effect on the female sex organization and furnishes additional evidence in favor of hormone activity during the process of sex differentiation. Positive evidence of the existence of primary sex-differentiating hormones is limited to the male sex, for the introduction of female substances into the male system is without effect on growth of the testes. It has therefore been assumed, in connection with other species than pig, that, while the male produces a hormone, the primary action of which is inhibition of growth of the germinal epithelium, the female lacks a comparable hormone, and typical ovarian growth takes place purely under zygotic stimulus. Accordingly, introduction of female substances into the male system is without effect, while introduction of male substances into the female will succeed in their overcoming zygotic influence and suppressing growth of cortex.

Although on the whole the assumption is applicable to the pig, it must receive some modification, as the situation differs somewhat from that in cattle. In cattle, introduction of the male stimulus succeeds in completely suppressing female growth, while in the pig, the germinal epithelium continues its activity even in face of the male hormone. This means either that the male sex-differentiating substances are unable completely to overcome zygotic factors in the female or that the female is elaborating some specific substance which has the effect of antagonizing or neutralizing male stimulus. We have seen that there is considerable variation in the degree of activity of the germinal epithelium in the modified gonads, in some there being a fair quantity of cortical cells present, while in others the germinal epithelium is practically reduced to a layer of flattened epithelium. The female element is therefore extremely variable. Any possibility of quantitative variation of the male stimulus as the cause of this variation, or even a time relation in the introduction of the male stimulus, must be ruled out of consideration, for the condition of the vascular anastomoses indicates that the most intimate connections, ones which had no doubt been formed very early in embryonic life, are not associated with the minimum of cortical substance in the female gonads, nor are cases in which the vascular connections are strongest and most abundant and the male hormone therefore quantitatively greatest (fig. 1, case C) associated with male dominance. It is not unlikely that this variation in degree of femaleness is merely an expression of ordinary individual variation; that individuals differ in the amount of resistance offered to outside influence purely on a zygotic basis, and that we are not dealing with antagonism of specific sex-differentiating hormones. On the other hand, there is indirect evidence from the normal interstitial content that possibly a specific female hormone is being elaborated at the commencement of sex differentiation. It is stated (Allen, '03) that in the pig interstitial cells appear in the ovaries in scanty quantities at the same time as they appear in abundance in the testes, although they shortly disappear in the ovary, not to reappear until toward the end of fetal life. If we accept the view that the interstitial cells are responsible for the hormone controlling the process of sex differentiation, a possible explanation arises to account for the difference in the two species, cattle and pig, for in the former species the interstitial cells do not appear in the developing ovary until late in fetal life, while in the testes they are produced at the commencement of sex differentiation (Bascom, '23). This condition in cattle supports the assumption of the precocity of male hormones, while in the pig the synchronous appearance of both male and female interstitial cells might account for the existence of both male and female stimuli. This argument must, however, be regarded as merely suggestive, for there is no conclusive evidence that the interstitial cells are responsible for the production of sex hormones even in the adult, and still less evidence is there that the same cells in the developing gonads produce the sex-differentiating hormones.

The existence of a specific female stimulus, if hormonic, might be expected, in addition to counteracting male influence in the female twin, to set up some sort of reciprocal reaction in the male twin. But in no case is there any evidence suggesting that any female stimulus is interfering in any way with normal testicular differentiation, for in no case can the testes of the male twins be distinguished from testes from single males of the same age. The scene of action, that is, antagonism between male and female stimuli, takes place in the female gonads only, and it is chiefly on these grounds that one might conclude that the counteracting female stimulus is of a zygotic nature, and not hormonic. This applies only to the primary gonadal stimulus, and not to any secondary stimuli which may arise in the gonads and control the secondary structures of the sex system.

A survey of the total number (seven) of pig freemartin-conditioned intergrades shows that there is considerable variation in the different cases. Generally speaking, the process of modification initiated by the introduction of male stimulus follows along two lines. Either it takes the form of a process of sex reversal or it tends to approach a negative or agonadic condition. In the former type the gonads are characterized by a minimal amount of cortex and by the well-differentiated medullary region; also, as far as could be determined, by the male type of growth of the reproductive ducts. Case no. 3 (previous paper) is of this type. The second type is represented by cases B and C. Here there is no dominance of either male or female elements; both cortical and medullary components of the gonads are very poorly represented, and this reduction is accompanied by a corresponding degree of underdevelopment or degeneration of both male and female ducts. It is to be noted that, although there is great reduction in the gonads of the second type, the actual quantity of cortical cells is greater than in the first type, and that this increase of female elements in the gonads is not only associated with a greater degree of female differentiation in the accessory apparatus, but also with a greater degree of inhibition of male growth in both gonads and ducts. From this it might be concluded that the specific male and female sex-differentiating stimuli, whether that of the female be hormonic or zygotic, are of an antagonistic nature, and that, unless one of them gains the upper hand, both are suppressed and rendered less effective. In some cases maleness becomes dominant and differentiation swings over in the male direction, while in case D, in which the male stimulus was withdrawn, femaleness has again gained the upper hand and sexual organization has become essentially female in structure. But where a balance of male and female stimuli is present and persists, neither maleness nor femaleness is able to express itself, and the agonadic condition results. There is, of course, the work on experimental hermaphroditism (Sand, '19, and Moore, '21) to indicate that the sex hormones of the adult are not of an antagonistic nature, for both testicular and ovarian transplants persist equally well in the same individual. But this is altogether different from the process of sex differentiation in the embryo, and there is no reason to suppose that the operation of the sex hormones is the same in the embryo as in the adult. The true hereditary hermaphrodites present a more serious objection, for in these both male and female elements must have developed equally well at the critical moment during sex differentiation for the gonads and ducts to have attained the complete intersexual condition exhibited in the adult. But here the situation is complicated by the existence of zygotic abnormalities, and we can hardly draw conclusions regarding the process of sex differentiation from such cases. Such a condition could not, I think, exist in cases where sex determination is normal as in the freemartin type of sex intergrades in the pig.

In cattle freemartins, also, there is considerable variation in the degree of intersexuality attained, and this is explained chiefly by the time factor (Lillie, '17, '23, and Bissonnette, '24). Cases exhibiting a high degree of transformation in the male direction may have practically complete internal structural organization of a male, including descended testes (without spermatozoa), vasa deferentia with associated glands and gonad connections. In these cases the male stimulus is supposedly introduced at an early date, inhibiting female growth from the commencement of sex differentiation. In other cases introduction of the male hormone is retarded and thus allows a certain amount of female growth to take place before modification sets in. Although proliferation of the germinal epithelium immediately ceases and degeneration of previously formed cortical substance takes place, portions of the müllerian ducts may persist and growth of the wolffian duct is not complete; the gonads usually retain the abdominal position and may either have the structure of testes or may be represented by an unorganized mass of tissue. The time relation in introduction of male stimulus is thus, in cattle, held responsible for the various grades of intersexuality and for the temporary existence of cortical substance in the gonads. It is also used as an explanation of the fragmentary condition of the reproductive ducts—a condition found to occur in some of the embryonic forms (Bissonnette, '21). In these it is assumed that normal female growth proceeded as far as the commencement of degeneration of the wolffian ducts, then, on introduction of the male stimulus, the process reversed and the müllerian ducts in turn commenced degeneration. Although the same condition, though perhaps to a greater degree, is found in the pig in cases herein described (B and C), the time explanation does not appear to be satisfactory. In the first place, the wolffian duct does not become intermittent until at least the 12-cm. stage, at which time the ovary is a large well-differentiated body and the müllerian ducts are also well developed. It is difficult to imagine the ovary and the ducts shrinking so suddenly as to become the minute structures of the 15-cm. embryo (cases B and C). Moreover, inasmuch as the degree of development of the müllerian ducts varies directly with the amount of cortical substance present in the gonads, while differentiation of the wolffian ducts varies likewise, it is more probable that the strength of the female stimulus rather than any time relation in male hormone introduction is the principal factor in determining the total course of differentiation pursued.

The fate of the reproductive ducts and associated structures is evidently closely related with gonad differentiation. The fact that growth of the müllerian ducts in the modified females varies in proportion with the amount of cortical substance present suggests that the stimulus required for their growth is located in the cortex. If we regard the unusual growth of the müllerian duct in the male twin of case B as due to female influence, then we must conclude that the cortex of the ovary is elaborating a specific hormone to stimulate growth of secondary structures, which hormone, circulating in the blood stream, reaches the male and stimulates growth of the müllerian ducts there. This single case is not sufficient, however, to warrant any conclusions, for, as previously stated, this irregularity might be a pure coincidence, and further proof is required before concluding that the female in any way succeeds in influencing male growth. There is, moreover, a great deal of evidence to indicate that growth of the genital ducts is controlled chiefly, if not wholly, by the gonads on the corresponding side. Numerous cases of hermaphroditism are described in mammals and birds in which bilateral asymmetry of the gonads is accompanied by a corresponding degree of asymmetry in the ducts (Kingsbury, '09, and Riddle, '21). In cases of adult pig hermaphrodites the interrelation between gonads and ducts is well demonstrated, for the degree of development of müllerian and wolffian ducts is, as a rule, directly dependent on the presence of ovarian and testicular substance in the corresponding gonad. Investigation into the developmental history of such cases would be highly desirable, especially as regards the presence of interstitial cells, and the correlation of gonadal elements with differentiation of the genital ducts during the critical moment for their development. A comparison of developmental stages of these hermaphrodites with the freemartin type of intergrade should serve to throw considerable light on the problem of sex determination and sex differentiation in the pig. Unfortunately, the desired material is rare and exceedingly difficult to procure, and there is no way at present of attacking the problem by experimental methods. The study of the freemartin condition in the pig must, moreover, be confined to the fetus, and we have no opportunity of seeing the final outcome of the process as it would appear in the adult. The cases described in this paper indicate that we might expect the adult to take on a variety of forms, including intergrades of the male type with descended testes; or forms in which the sex organs are greatly reduced or absent; or again, possibly, the hermaphrodite type with ovotestes. The gonadless forms, or those with greatly reduced gonads, have not been described in the adult pig, although they are known to occur in cattle. A more complete record of sexual abnormalities in the pig might bring such types to light. Several cases of the male type of intergrade have been described, and, apart from those that are known to be true zygotic hermaphrodites, it is possible that a considerable number of them are the result of the freemartin condition.

This report forms part of the subject-matter for a thesis to be submitted for the degree of Ph.D. at The University of Chicago. I would like to express my thanks to Dr. H. H. Newman, who is supervising the thesis, for helpful criticism throughout the work.

## CONCLUSIONS

1. The intermingling of male and female blood during embryonic life in the pig results in modification of the internal genital system of the female and produces a form of sex intergrade comparable with the cattle freemartin.

2. Female differentiation is not completely suppressed as in cattle, for the germinal epithelium retains its activity to a certain degree even in the face of the male hormone.

3. Differentiation of the sex organization of this type of sex intergrade is concluded to be directed under the antagonistic influence of male and female stimuli.

4. The intensity of the female stimulus, as determined by the amount of cortex, varies in the different cases. A minimal amount of female stimulus presumably is not sufficient to counteract male influence, and differentiation therefore takes place along male lines. A stronger female stimulus inhibits male growth, and male, as well as female, elements of gonads and ducts are in a greatly reduced condition.

5. Variations in intensity of female stimulus are probably zygotically determined.

6. Withdrawal of male influence before sex differentiation is completed allows return of normal female growth.

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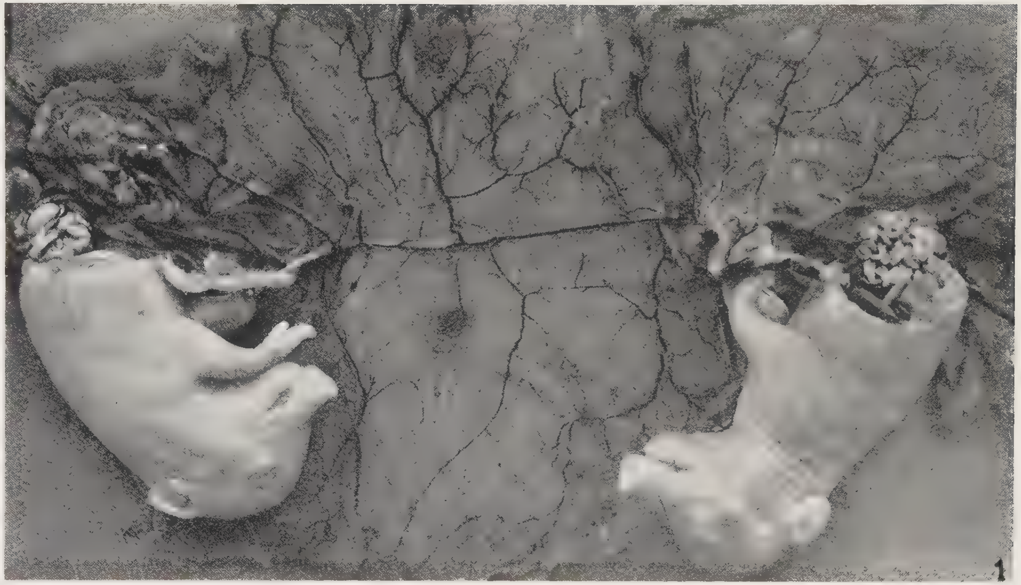
## ABBREVIATIONS

*B.u.gland,* bulbo-urethral gland
*B.v.,* blood vessels
*Epooph.,* epoöphoron
*Epidid.,* epididymis
*G.,* gonad
*G.c.,* genital cord
*G.e.,* germinal epithelium
*Gen.rd.,* genital ridge
*Gub.,* gubernaculum
*Mes.,* mesonephros
*Mes.d.,* mesonephric duct
*M.f.,* mesonephric fold
*Met.,* metanephros
*M.D.,* or *Müll.d.,* müllerian duct
*Ost.,* ostium
*P.c.,* primary sex cords
*Pros.ura.,* prostatic urethra
*R.,* rete
*R.lig.,* round ligament
*S.c.,* secondary sex cords
*S.t.,* seminiferous tubules
*S.vag.,* saccus vaginalis
*Sem.ves.,* seminal vesicles
*T.a.,* tunica albuginea
*Um.,* umbilicus
*Um.art.,* umbilical artery
*Ura.,* urethra
*Urog.s.,* urogenital sinus
*Ut.,* uterus
*Ut.mas.,* uterus masculinus
*Vest.,* vestibule
*V.def.,* vas deferens
*W.D.,* wolffian duct

## PLATE 1

### EXPLANATION OF FIGURES

Photograph showing monochorionic twins and fetal membranes. Circulation injected from male (right) umbilical cord. Veins show dark; arteries, pale.

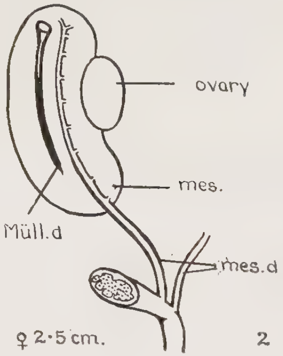

1

## PLATE 2

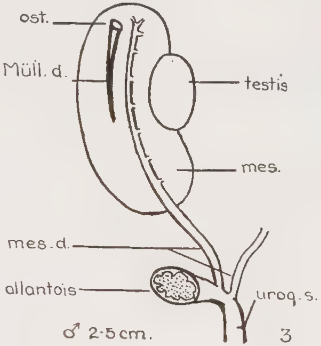
### EXPLANATION OF FIGURES

Reconstructions from serial sections, showing normal development of the urogenital system in the pig.

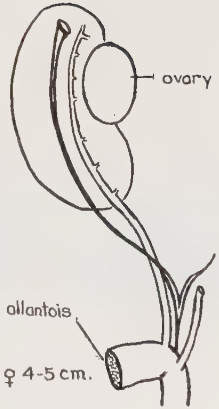
PLATE 2


ovary
mes.
Müll.d
mes.d

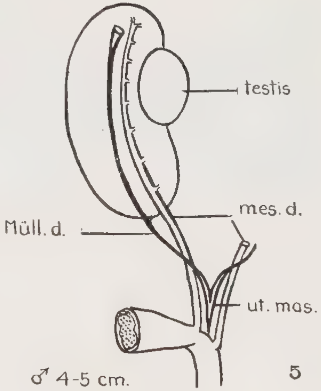
♀ 2·5 cm. 2


ost.
Müll. d.
testis
mes.
mes. d.
allantois
uroq. s.

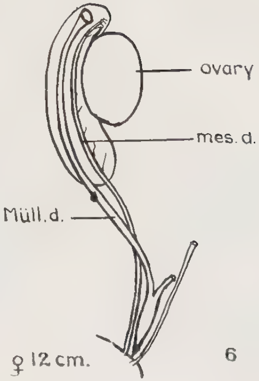
♂ 2·5 cm. 3


ovary
allantois

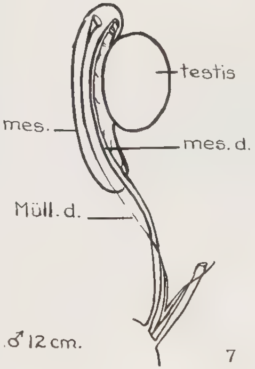
♀ 4-5 cm. 4


testis
Müll. d.
mes. d.
ut. mas.

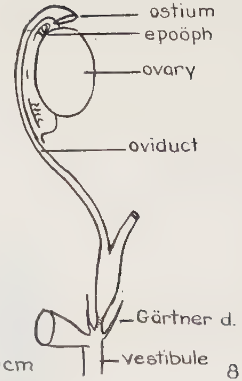
♂ 4-5 cm. 5


ovary
mes. d.
Müll. d.

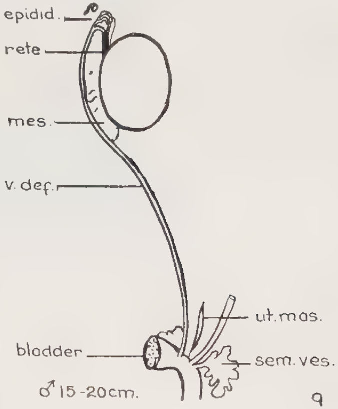
♀ 12 cm. 6


testis
mes.
mes. d.
Müll. d.

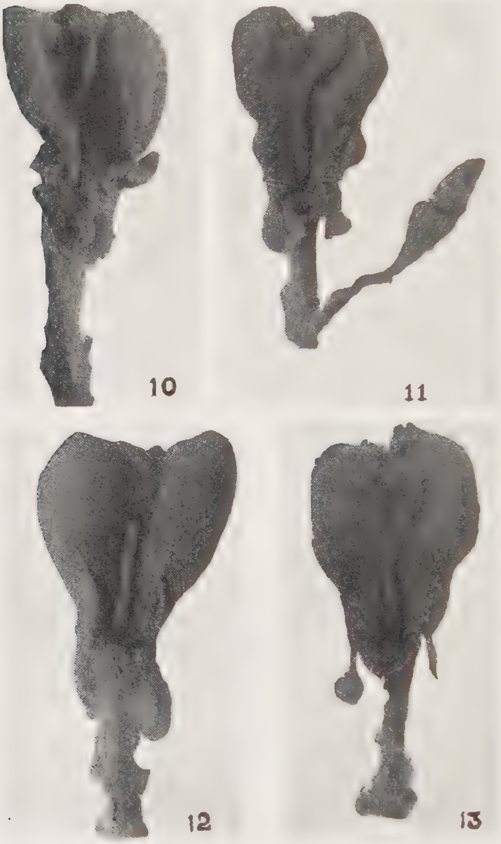
♂ 12 cm. 7


ostium
epoöph
ovary
oviduct
Gärtner d.
vestibule

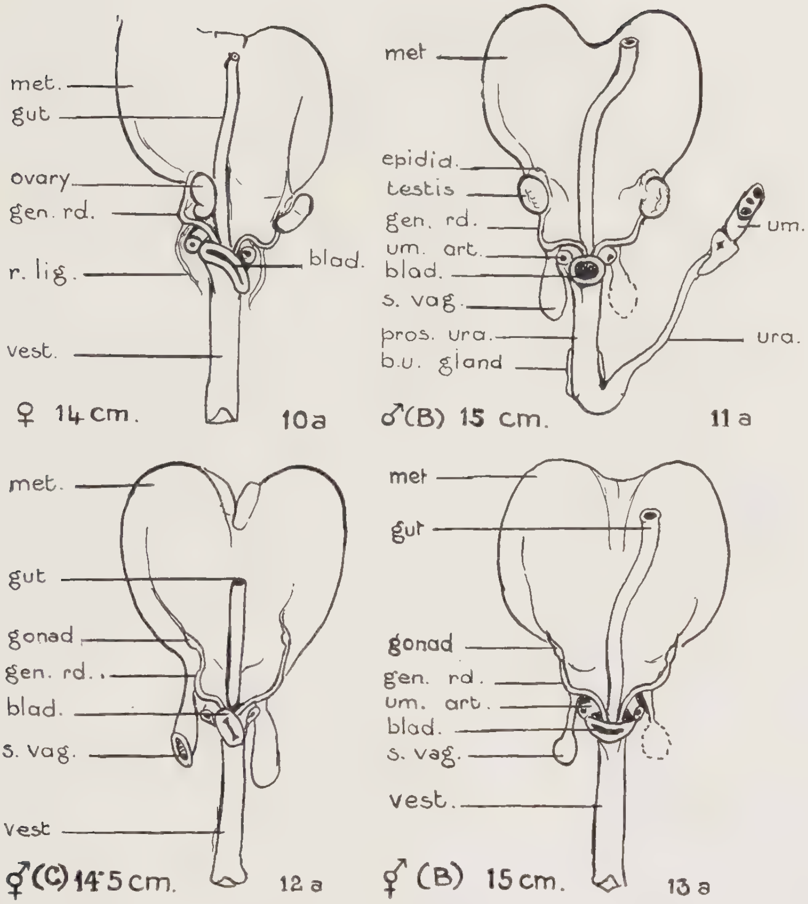
♀ 15-20cm 8


epidid.
rete
mes.
v. def.
ut. mas.
bladder
sem. ves.

♂ 15-20cm. 9



Photographs of dissected urogenital systems (see key, pl. 4).
10 Normal female, 14 cm.
11 Male twin, 15 cm., case B.
12 Female twin, 14.5 cm., case C.
13 Female twin, 15 cm., case B.

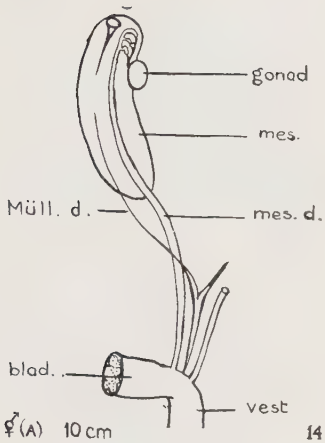

met.
gut
ovary
gen. rd.
r. lig.
blad.
vest.
♀ 14 cm.
10a
met
epidid.
testis
gen. rd.
um. art.
blad.
s. vag.
pros. ura.
b.u. gland
um.
ura.
♂(B) 15 cm.
11 a
met.
gut
gonad
gen. rd.
blad.
s. vag.
vest
⚥(C) 14·5 cm.
12 a
met
gut
gonad
gen. rd.
um. art.
blad.
s. vag.
vest.
⚥ (B) 15 cm.
13 a

10a, 11a, 12a, 13a Keys to figures 10, 11, 12, 13, respectively.

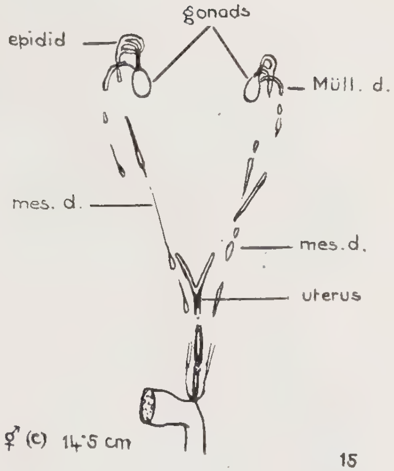
## PLATE 5

### EXPLANATION OF FIGURES

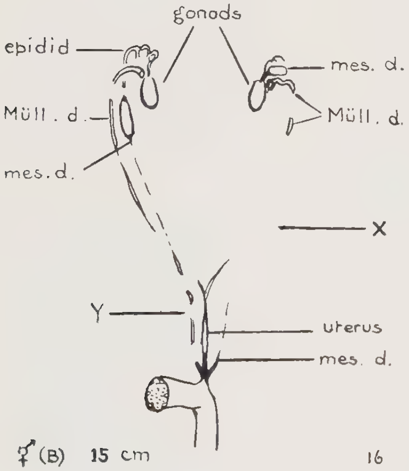
Reconstructions from serial sections, showing abnormal development of the urogenital system.


gonad
mes.
Müll. d.
mes. d.
blad.
vest
⚥(A) 10 cm

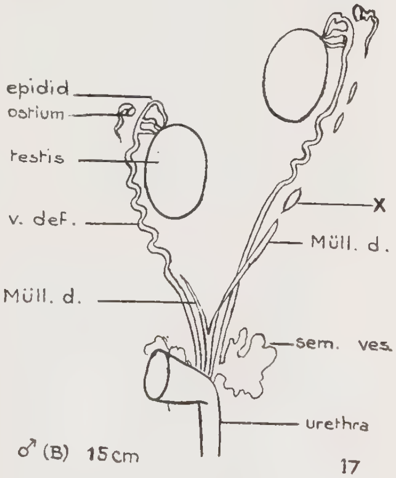
14


gonads
epidid
Müll. d.
mes. d.
mes. d.
uterus
⚥ (C) 14·5 cm

15


gonads
epidid
mes. d.
Müll. d
Müll. d.
mes. d.
X
Y
uterus
mes. d.
⚥ (B) 15 cm

16


epidid
ostium
testis
v. def.
Müll. d.
X
Müll. d.
sem. ves.
urethra
♂ (B) 15 cm

17

## PLATE 6

### EXPLANATION OF FIGURES

18 Photomicrograph of section through normal testis of 11-cm. embryo. × 35.

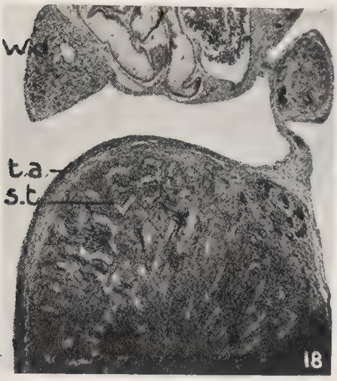
19 Photomicrograph of section through normal ovary of a 14-cm. embryo. × 35.

20 Photomicrograph of section through gonad and mesonephric folds of 15-cm. modified female, case B. × 35.

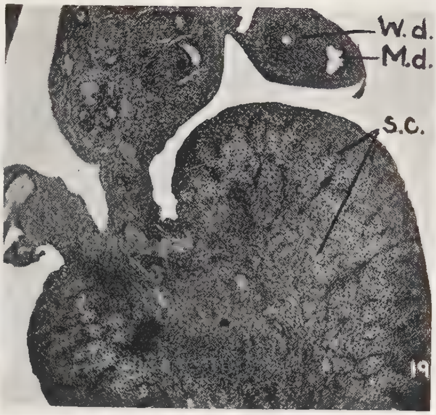
21 Photomicrograph of section of gonad of 15-cm. modified female, case D. × 35.

22 Photomicrograph of section of gonad of 14.5-cm. modified female, case C. × 35.

23 Photomicrograph of section through gonad of 10-cm. modified female, case A. × 35.



18



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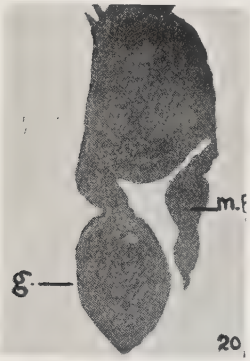
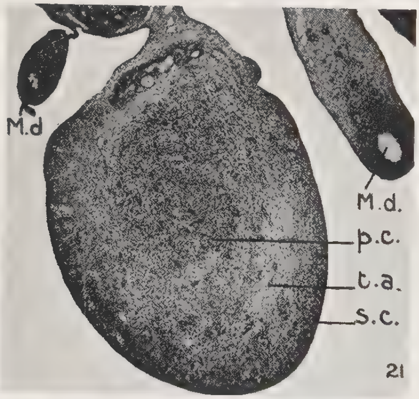
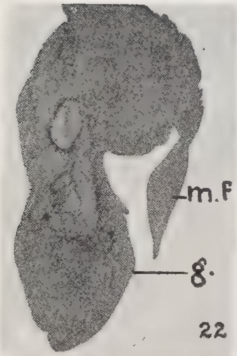


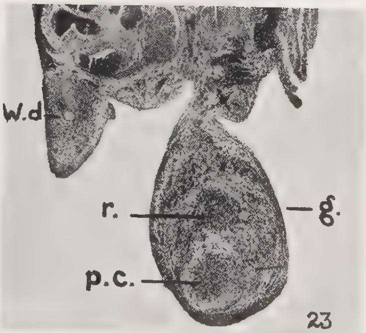
20


M.d
M.d.
p.c.
t.a.
s.c.

21



22



23

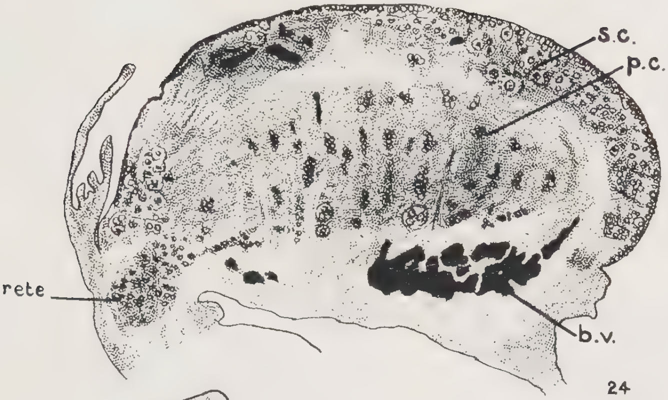
## PLATE 7

### EXPLANATION OF FIGURES

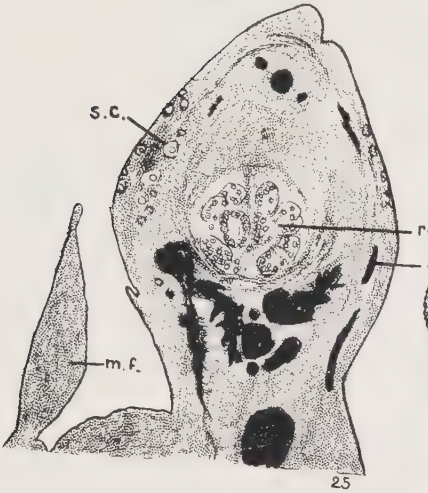
24 Diagram of longitudinal section through ovary of 15-cm. modified female, case D.

25 Diagram of cross-section of gonad of 14.5-cm. modified female, case C.

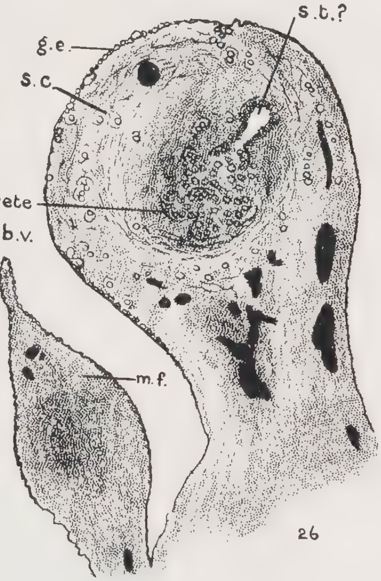
26 Diagram of cross-section of gonad of 15-cm. modified female, case B.


s.c.
p.c.
rete
b.v.

24


s.c.
rete
b.v.
m.f.

25



26

## PLATE 8

### EXPLANATION OF FIGURES

27 Photomicrograph of section through genital cord of normal female embryo, 14 cm. × 35.

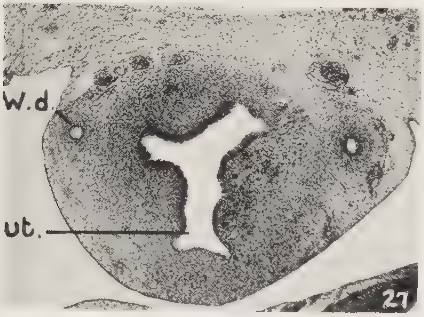
28 Photomicrograph of section through same region of 15-cm. modified female, at level indicated by letter *Y* in figure 16. × 35.

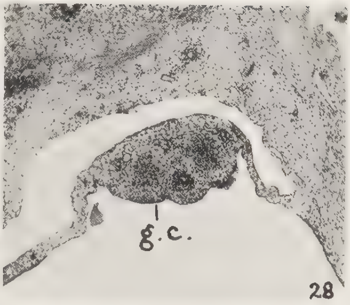
29 Photomicrograph of section through genital ridges of normal female, 14 cm. × 35.

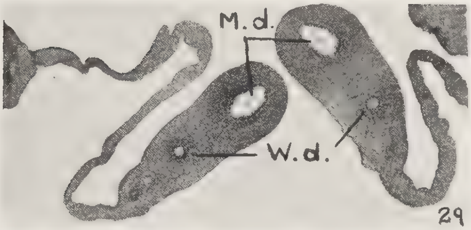
30 Photomicrograph of section through same region of 14.5-cm. modified female, case C. × 35.

31 Photomicrograph of section through genital ridges of male twin, 15 cm., case B. × 35.

32 Photomicrograph of section through genital ridges of 15-cm. modified female, case B. × 35.


W.d.
ut.
27


g.c.
28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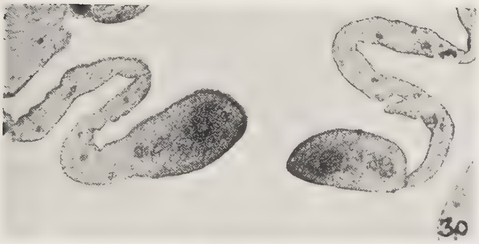
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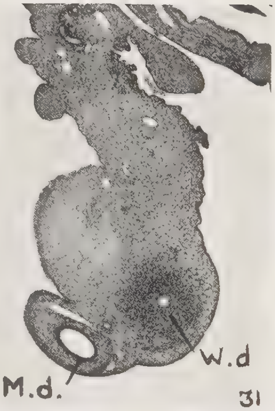
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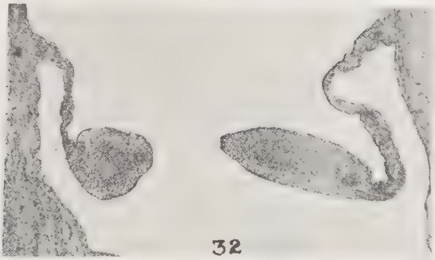
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# PROCEEDINGS OF THE TWENTY-SIXTH ANNUAL MEETING OF THE AMERICAN SOCIETY OF ZOÖLOGISTS

NEW YORK, NEW YORK, DECEMBER 27, 28, 29, 1928

The 1928 meeting of the American Society of Zoölogists was held at Teachers College, Columbia University, New York City, December 27th, 28th, and 29th, in conjunction with the other societies affiliated with Section F of the American Association for the Advancement of Science. The local committee is deserving of great credit for providing adequate accommodations for the interlocking sectional programs and for the special features which added greatly to the interest of the occasion.

Sessions for the reading of papers were unusually well attended, in some instances the attendance approaching two hundred. As in recent years, the presentation of papers by demonstration was featured; this method of presentation undoubtedly adds greatly to the interest and value of contributions.

Several special features added to the interest of the meetings. Thursday noon, the Society provided transportation by buses to the New York Zoölogical Park, where a complimentary luncheon was furnished by the New York Zoölogical Society; in the afternoon, a supervised tour of inspection of the park was taken. On Thursday evening, a zoölogical smoker was held at the American Museum of Natural History. The appropriate setting of the museum rooms enhanced the pleasure of the occasion; an additional attraction was the showing of motion pictures of subtropical marine life taken under water by Dr. William Beebe. On Saturday afternoon and evening, the Society gave precedence to the symposium and banquet of the American Society of Naturalists.

Among the many additional features, three motion pictures were specially scheduled: one was "The Story of Antivenin," exhibited late Thursday afternoon; a second was the famous Canti film, showing the activities of living cells in vitro, demonstrated late Friday afternoon before a large audience in the Horace Mann Auditorium and appropriately explained by Prof. C. A. Kofoid; the third was a special showing of motion pictures depicting wild life, obtained during expeditions sponsored by the American Museum; these included such topics as the finding of dinosaur eggs on the Gobi Desert Expedition and the latest unreleased films of African mammals taken by Mr. and Mrs. Martin Johnson.

All members of the Executive Committee were present except Dr. D. E. Minnich and Dr. M. F. Guyer, both of whom are abroad. The resulting quorum made possible the first meeting of the Committee which has been held in several years.

## INTRODUCED PAPERS

By consent of the Society, the following papers were introduced:

### A Case of Cyclic Change in Parathyroid Tissue

ROY A. WAGGENER
*Carleton College*

(Introduced by B. F. Kingsbury)

*Read*

The parenchymal cells in the parathyroids of Rana catesbeiana (Shaw) are found to undergo a very curious cyclic change. The cytoplasm of the cell is broken down by a process of cytolysis which first causes the cells to appear vacuolized after acid fixation. The nuclei are decomposed by chromatolysis and pycnosis; and the entire parenchyma, except for a layer of cells lining the capsule, is finally liquefied. During a stage in this process the products of liquefaction may be preserved and demonstrated to be in the form of a colloid.

The parathyroid body is reconstructed by young cells which are produced from rather definite growth centers. These regeneration processes begin some time before cytolysis of the old tissue is completed and continue until the entire organ is restored. Mitotic figures are quite common during this period of rapid growth. The cytolytic and regenerative processes transpire in such sequence that the amount of apparently healthy tissue present at any one time is a rather large proportion of the total mass normally present. The relationship of the parenchyma to the connective tissue and vascular elements is described.

### THE DEER-MOUSE, PEROMYSCUS, CAN BE MADE A VALUABLE LABORATORY MAMMAL

GEORGE T. MC NAIR
*University of Kansas*

(Introduced by H. H. Lane)

*Read*

It is not generally recognized that the deer-mice of the genus Peromyscus represent an excellent laboratory mammal, probably better than any other wild mammal in this country.

Species of this genus are to be found in abundance in every section of the United States. They are easily caught and handled, are cheaply housed and fed. They are exceptionally free from disease. They are prolific, and they start breeding at a little over two months of age. With proper treatment, a large proportion will produce young every month in the year, while subspecies and even different species will interbreed freely in captivity. The writer catches these mice in a simple trap consisting of a tin can wired onto an ordinary spring mouse trap with a piece of hail screen fastened to the spring to close the mouth of the can. After three years of experimenting with their care, the writer is able to get a higher rate of reproduction from these mice in the laboratory than occurs in the native wild state. This is shown effectively by graphs. The essentials for their care consist of five simple requirements:

1) A comparatively quiet room for cages. 2) A relatively low temperature and subdued light in the room. 3) A proper diet; a mixture of oats, oatmeal, freshly sliced carrots, and occasional bone scraps is the most effective and convenient. 4) Overfeeding must be guarded against. The writer feeds only every other day. 5) Cleanliness.

### DEMONSTRATION OF THE EFFECT OF HIGH-FREQUENCY SOUND WAVES ON CELLS

E. NEWTON HARVEY
*Princeton University*

*By demonstration*

### PRESSURE PHENOMENA IN THE DIVIDING OYSTER EGG

W. F. WELLS

(Introduced by C. B. Davenport)

*By demonstration*

### CILIARY MOVEMENT

J. GRAY
*Columbia University*

(Introduced by E. B. Wilson)

*By demonstration*

## Effect of Sympathectomy on Visceromotor Reflexes

ALBERT KUNTZ AND ALVER H. KERPER
*St. Louis University School of Medicine*

*By title*

That reflex movements of the hind limbs may be elicited by stimulation of the mesenteric nerves or abdominal viscera in decapitate animals is well known. In a series of cats in which the spinal cord was transected at the level of the foramen magnum following unilateral extirpation of the lumbar and upper sacral portion of the sympathetic trunk, electrical and mechanical stimulation of the mesenteric nerves or abdominal organs regularly elicited reflex movements in the hind limb on the side on which the sympathetic trunk remained intact, but rarely in the hind limb deprived of its sympathetic innervation. Visceromotor reflexes involving this limb could only be elicited by visceral stimulation which was much stronger than that required to elicit fairly vigorous reflex responses in the normally innervated limb. Although contraction of skeletal muscles cannot be elicited by sympathetic stimulation alone, it seems not improbable that the irritability of the visceromotor reflex mechanism is diminished by sympathetic denervation of the muscle involved.

## Chromosomal Variation correlated with Geographical Distribution in Circotettix verruculatus (Orthoptera)

EDWIN R. HELWIG
*University of Pennsylvania*

(Introduced by C. E. McClung)

*By title*

The first spermatocytes of Circotettix verruculatus have eleven chromosomes. Five of these are regularly atelomitic and three are telomitic. The other three are variable and may have both diads telomitic, both atelomitic, or one diad telomitic while the other is atelomitic. The locus of fiber attachment, and, consequently, the form of the chromosome are constant for the individual. The point of fiber insertion is known to be inherited according to mendelian principles, and is, therefore, a measure of the frequencies of the telomitic and atelomitic conditions in a group of individuals.

Samples of five geographically different populations were studied with respect to the proportion of atelomitic to telomitic diads in the three variable tetrads. The proportion for each of the three chromosomes was compared with that for the corresponding tetrads from the other localities. The data were subjected to statistical analysis, and significant differences between some of the groups were found in the proportion of atelomitic to telomitic diads in corresponding chromosomes.

A possible correlation between these cytological differences among the various localities and the formation of geographical races or subspecies is discussed. An inversion of the portion of a chromosome, which causes an apparent, although not a real, change in the locus of fiber attachment is suggested as the origin of atelomitic chromosomes. The effect which heteromorphism of synaptic mates may have on crossing-over is considered.

### Hermaphroditism in the Bullfrog

F. E. CHIDESTER
*West Virginia University*

*By title*

Since reporting two cases of incipient hermaphroditism in the bullfrog, in which diffuse egg masses were found attached to the mesentery and viscera of otherwise normal male frogs, the writer has secured from a single lot of young bullfrogs five cases of more advanced hermaphroditism. In two of the specimens a testis was found on the right side and an ovary on the left, while in the other three specimens testes and ovarian masses were both present, the eggs being separate from the testis in each instance. Rudimentary oviducts were not present.

### Binary Fission and Induced Encystment and Excystment in Diophrys hopkinsii, sp.nov.

AGNES PLATE AND WALDO FURGASON
*Stanford University*

(Introduced by C. V. Taylor)

*By title*

Diophrys, a newly described marine ciliate, has been successfully cultured. Its form and size, which may vary considerably, together with its prominent locomotor organelles, afford unusual opportunity for detailed observation and experimental studies. Binary fission can be readily followed throughout the process. Both encystment and excystment have been induced at will. Reencystment and reexcystment have successfully been induced, but with diminishing capacity. Encystment may be induced more readily during the day and in more mature individuals rather than in newly divided ones. Reorganization occurs during binary fission, but apparently does not occur during encystment. Parasitism by an unknown flagellate has frequently been observed and is described.

### Evolutionary Trends in the Genus Spirifer

CARROLL LANE FENTON
*University of Cincinnati*

(Introduced by W. C. Allee)

*By title*

In the brachipod genus Spirifer there are several pronounced evolutionary trends involving characters of the shell as a whole and of surface ornamentation. In the S. varians and related gentes, two of these trends are closely correlated. There is no indication of heterogenetic change in the characters which they involve; they reappear in successive subgentes and even species, at various stratigraphic levels and without discernible relationship to the environment. They are minutely reproduced in ontogeny by the repetition of adult stages, and, phyletically as well as ontogenetically their advanced development is terminated by death. This close parallel between individual development and racial supports the theory of racial senescence whose basis is identical with that of senescence in the individual.

### EXPERIMENTAL EVIDENCE THAT THE ROTATION OF THE CRYSTALLINE STYLE IS NOT A SUBSTITUTE FOR GASTRIC PERISTALSIS IN THE CLAM (SCHIZOTHAERUS NUTTALLII)

T. L. PATTERSON
*Detroit College of Medicine and Surgery and Hopkins Marine Station, Stanford University*

(Introduced by Charles W. Creaser)

*By title*

Studies made on the peristaltic activity of the stomach of the gaper clam by the balloon method before and after surgical removal of the crystalline style demonstrates unequivocally that the rotation of the style is not a substitute for gastric peristalsis. This is in contradistinction to the view of Nelson, who maintains that the style, among other things, functions in this way. The gastric contractions after removal of the style are practically identical with those obtained when it is intact, and in many animals they even become more vigorous in its absence. These contractions are continuous both in the presence and in the absence of the style, and they show a definite relationship to the gastric activity observed in various gastropods, which possess no crystalline style, namely: Haliotis rufescens (Abalone); Cryptochiton stelleri (Chiton); Ariolimax californicus (giant slug), and Tethys californica (sea hare). The contractions are not due to muscular movements of the foot, since they go on uninterrupted when the foot is quiescent, as is determined graphically by simultaneous records taken from the stomach and the foot. Therefore, the musculature of the stomach of this animal produces the gastric contractions which places it in the same physiological category as the stomachs of other vertebrate and invertebrate animals studied.

## PROGRAM

Sixty-nine papers were scheduled to be read, exclusive of those on joint programs. Fifty-six of these were read, the remaining thirteen being read by title. Sixty-four other papers were scheduled by title. Thirty-six papers were scheduled by demonstration or exhibit, nearly all of which were presented. In the following summary the papers are designated by their program numbers (compare December number of this Journal).

### THURSDAY, DECEMBER 27TH

Section a. 10.00–12.15 Caswell Grave, president, presiding.

*Comparative* and *General Physiology* (Animal Reactions and Sensory Physiology). Papers 1 to 12, inclusive; nos. 3, 4, 6, and 9 were read by title, owing to the absence of the authors.

Section b. 10.00–12.15 H. W. Stunkard and L. B. Arey, acting chairmen, presiding.

*Protozoölogy, Comparative Anatomy,* and *Miscellaneous,* concluded. Papers 78 to 81 and 91 to 94, inclusive.

1.00–2.00 Luncheon and inspection of New York Zoölogical Park.

2.00–4.00 Demonstrations and exhibits.

3.00–4.00 Motion-picture demonstrations.

4.30 Motion picture, ''The Story of Antivenin.'' Horace Mann Auditorium.

9.30–11.30 Zoölogists' Smoker, American Museum of Natural History.

### FRIDAY, DECEMBER 28TH

Section a. 9.00–12.00 Caswell Grave, president, presiding.

*Cytology* and *Embryology,* concluded. Papers 115 to 121 and 135 to 145, inclusive; nos. 118, 120, 135, and 139 were read by title, owing to the absence of the authors.

Section b. 10.00–12.00 Joint session with the American Society of Parasitologists.

1.30–3.30 Caswell Grave, president, presiding.

*Comparative* and *General Physiology* (Endocrinology). Papers 13 to 21, inclusive.

3.30–3.45 Business session of Section F.

3.45–4.30 Business session of the American Society of Zoölogists.

4.30–5.30 Motion picture: Canti film of living cells in vitro. Dr. C. A. Kofoid. Horace Mann Auditorium.

7.00 Zoölogists' dinner, Dr. Caswell Grave presiding. The address was given by Dr. C. E. McClung, retiring vice-president of Section F, on ''The nature of biological papers and the obligations of their authors.'' About two hundred and twenty-five attended the dinner.

### SATURDAY, DECEMBER 29TH

Section a. 9.00–12.00 Caswell Grave, president, presiding.

*Comparative* and *General Physiology* (Biochemical subjects). Papers 22 to 39, inclusive; nos. 24, 25, 28, 30, and 39 were read by title, owing to the absence of the authors. Several introduced and deferred papers were read at the close of this session.

Section b. 10.00–12.00 Joint session with the Ecological Society of America.

## REPORTS OF OFFICERS AND COMMITTEES

### *Acting Secretary's Report*

The report of the Acting Secretary showed the roster of membership to be as follows: At the beginning of 1928 there were 6 life members, 443 active members, and 57 associate members, making a total membership of 506. Three active members, H. H. Wilder, C. O. Esterly, Emily R. Gregory) and 1 life member (Bashford Dean) died during the year. Two members (A. Weinstein, H. Roxas) were dropped for non-payment of dues. Later in the meeting there were elected 17 new active members, 4 associate members to active membership, 1 member reinstated to active membership, and 31 new associate members. This brings the total membership at the beginning of 1929 to 549.

The report of the Acting Secretary was adopted and ordered filed.

### *Treasurer's report*

*Report of the Treasurer of the American Society of Zoölogists for the year 1928*

| | | |
|---|---|---|
| *Credits:* | | |
| Balance on hand December 24, 1927, when accounts were last audited | $1306.24 | |
| Receipts from dues, 1928 | 992.70 | |
| Interest on deposits (July 1, 1927, to July 1, 1928) | 33.82 | |
| | $2332.76 | $2332.76 |
| *Expenditures:* | | |
| Secretary's expenses: | | |
| Traveling and incidental expenses at annual meeting | $172.31 | |
| Stenographer | 134.38 | |
| Printing | 161.41 | |
| Stamps, telegrams, and express charges | 37.59 | |
| | $505.69 | |
| Treasurer's expenses: | | |
| Exchange on checks | $20.35 | |
| Stamps, stationery, and printing | 91.62 | |
| Stenographer | 36.57 | |
| The Wistar Institute: | | |
| Anatomical Record programs | 260.98 | |
| Genetics dues forwarded to Genetics Treasurer | 2.50 | |
| Total Treasurer's expenditures | 412.02 | |
| Total Secretary's expenditures | 505.69 | $ 917.71 |
| Balance on hand December 21, 1928, deposited in the name of the American Society of Zoölogists in the National Bank of the Republic, Chicago, Illinois | | $1415.05 |

Respectfully submitted,

L. B. Arey, *Treasurer.*

The report of the Treasurer was accepted and ordered filed.

The Auditing Committee, consisting of Edward L. Rice and J. Paul Visscher, reported the Treasurer's books to have been examined and to have been found correct. Their report was accepted and ordered filed.

Through the Acting Secretary, Dr. S. I. Kohnhauser, representative of the Society on the Executive Committee of the Commission for the Standardization of Biological Stains, presented the following report for 1928:

*To the Officers and Members of the American Society of Zoölogists:*

As your representative on the ''Commission for the Standardization of Biological Dyes,'' I wish to report the following progress for the year 1928. A meeting of the Executive Committee was held in Washington, D. C., on April 30th, at which time we discussed plans for the work of the year.

A few of the most useful dyes have been added to the large list already certified, and an increasing number of samples bearing the certification label are now on the market. It is significant to find that the companies submitting the dyes are now sending in samples which are generally satisfactory. Whereas, in 1923, 45 per cent of the samples submitted for certification were rejected as unsuitable for biological work, in 1928, only 3 per cent of the samples submitted were rejected by the Commission. It thus appears that the companies have used greater care in selecting their products in accordance with the specifications laid down by the Commission. The process, therefore, of educating the manufacturers and jobbers as to the needs of the biologist has been well accomplished. This means a considerable saving of time and money. The Commission itself, which employs a full-time technician at Geneva, is now equipped to carry on the tests with much less delay than heretofore. This is especially gratifying to the manufacturers and jobbers.

Your representative has assisted in the testing of eosin bluish, sudan IV, Wright's blood stain, neutral red, and Janus green.

''Biological Stains'' has been revised, and a new edition will probably be published during the coming year. ''Stain Technology'' has been received well on the part of biologists, and practically all original subscribers have continued to take this journal. Both of these publications are now on a self-paying basis, although no advertising has been taken. Members of the Society of Zoölogists are urged to send in articles dealing with the use of biological dyes. They will be assured an early publication.

Members are especially urged to notify their representative in case any certified dye fails to meet the requirements for which the dye is intended to be used. We would be very glad, also, to receive any suggestions concerning improvements of dyes or additions to the certified list.

Respectfully submitted,
S. I. Kornhauser.

The Necrology Committee submitted minutes on the deaths of three members which occurred during the past year. The minutes, adopted by a rising vote, were ordered spread on the records and copies sent to the families of the deceased.

### *Resolution on the life and work of Harris Hawthorne Wilder*

By the death of Prof. Harris Hawthorne Wilder, of Smith College, the American Society of Zoölogists has lost one of its most gifted and distinguished members. Professor Wilder was President of the Society in 1922.

He was born in Bangor, Maine, April 7, 1864, the only child of Solon Wilder, a musical director, and Sarah Wilder, the daughter of a physician. The Wilder family is of English ancestry. The first paternal ancestor arrived at Hingham in 1638. Doctor Wilder's mother was of mixed English, Scotch, and Irish stock.

As a student at Amherst College he came under the inspiring influence of Dr. John M. Tyler, who strengthened the naturalistic bent which had been manifest early in his life. After graduation, he taught biology in the Chicago schools, and later took his doctor's degree at Freiburg under Wiedersheim and Weismann. In 1892, he became professor of zoölogy at Smith College, where he met and married Inez Whipple, another able zoölogist.

Among Doctor Wilder's best-known researches are those on ''Die Nasengegend von Menopoma,'' ''Lungenlose Salamandriden,'' ''Duplicate twins and double monsters,'' ''The morphology of cosmobia.'' His most important books are his ''History of the human body'' (1910 and 1923) and ''The pedigree of the human race'' (1926), both of which are works of marked originality and reveal the pioneering quality of Wilder's mind. His remarkable researches on the epidermic markings of palms and soles, carried on in collaboration with Mrs. Wilder, have stimulated morphological, genetic, and anthropological investigations in all parts of the world.

Doctor Wilder's personal qualities endeared him to all who knew him. He was modest, gifted with a keen sense of humor, and loyal as a friend. His many students remember him as a most inspiring teacher.

Be it therefore,

*Resolved,* That in his untimely death this Society has suffered an irreparable loss;

That the Society express to Inez Whipple Wilder its deep sympathy;

And that the Secretary be authorized to spread these resolutions upon the minute-book.

HERBERT V. NEAL.

### *Resolution on the life and work of Calvin O. Esterly*

The American Society of Zoölogists records with regret the recent loss of an honored member, Prof. Calvin O. Esterly, who died at La Jolla, California, August 10, 1928. Doctor Esterly was an alumnus of the University of California and a doctor of philosophy of Harvard University. At the time of his death he was professor of zoölogy at Occidental College and a non-resident member of the research staff of the Scripps Institution of Oceanography.

To his fellow scientists Esterly was chiefly known for his extensive and fruitful studies of the marine Copepoda of the Pacific coast, studies which dealt alike with the taxonomy, ecology, and behavior of this group of organisms. His considerable bibliography in this field culminated in what is perhaps his most important contribution, ''The periodic occurrence of Copepoda in the marine plankton of two successive years at La Jolla, California.'' This was published a few months before his death.

As a teacher, Professor Esterly had the affectionate regard alike of his students and his colleagues on the faculty. His friends unite in bearing testimony of his modesty, simplicity of character, high sense of duty, and lovable nature.

*Resolved,* That a copy of this memorial be forwarded to Mrs. Ruth Esterly and that the Secretary record these resolutions among the minutes of this meeting.

F. B. SUMNER.

### *Resolution on the life and work of Bashford Dean*

We, the officers and members of the American Society of Zoölogists, wish to place on record in the form of this brief sketch and minute our sorrow and deep sense of loss in the untimely death of our colleague Dr. Bashford Dean.

Doctor Dean was born in New York City on October 28, 1867, and died on December 6, 1928, at Battle Creek, Michigan, at the age of sixty-one. His people on both sides were of old English and Holland Dutch stock, who were established in the region of New York City long before the American Revolution.

He graduated from the College of the City of New York in 1886 at the age of nineteen and was immediately appointed tutor in zoölogy. Shortly thereafter, he entered Columbia College, where he studied geology and fossil fishes under Dr. J. S. Newberry, and took his Ph.D. degree in 1890, at the age of twenty-three. Following graduation, he was successively instructor, adjunct professor, and professor of vertebrate zoölogy in Columbia. During this time he became the leading American student of the palaeontology, embryology, and morphology of fishes, especially the ancient shark-like fishes and the present-day ganoids.

From 1903 to 1910, Dean was curator of fossil fishes and then of ichthyology in the American Museum. During that time he installed the first habitat groups of fossil and recent fishes ever made in America. From 1910 till his death, he was honorary curator of ichthyology, but even then, though his main interests were in other lines of work, he helped through to publication the great three-volume ''Bibliography of fishes,'' which with his own hands he had begun many years before. For this he was awarded the D. G. Elliott medal in 1923.

With deep regret we record that in Doctor Dean's death science has lost an eminent man, ichthyology an outstanding figure, the American Society of Zoölogists a distinguished member, and many of us a personal friend.

Therefore, be it

*Resolved,* That this expression of our sorrow be incorporated in the minutes of this meeting and that copies be sent Mrs. Bashford Dean and Miss Harriet Dean.

E. W. GUDGER.

### ELECTION OF OFFICERS

The Nominating Committee (Art. III, Sec. 6), composed of F. R. Lillie, Charles Zeleny, and H. J. Muller, submitted the following report:

*President*, one year, C. B. DAVENPORT, Carnegie Institution.

*Vice-President*, one year, FERNANDUS PAYNE, Indiana University.

*Executive Committee*, five years, CASWELL GRAVE, Washington University.

*Council, A.A.A.S.*, one year (two to be elected), W. C. ALLEE, University of Chicago; P. W. WHITING, University of Pittsburgh.

*Editorial Board, Journal of Morphology and Physiology*, three years (three to be elected), S. R. DETWILER, Columbia University; W. J. CROZIER, Harvard University; W. W. SWINGLE, University of Iowa.

*Representative to Division of Biology and Agriculture of National Research Council*, three years, H. S. JENNINGS, Johns Hopkins University; *alternate*, L. C. DUNN, Columbia University.

*Type Culture Committee*, three years, C. A. KOFOID, University of California.

A new slate for the Editorial Board of the Journal of Morphology and Physiology was nominated from the floor; the new nominees were S. R. Detwiler, R. S. Lillie, and H. J. Muller. There being no other nominations, the Acting Secretary was instructed to cast a unanimous ballot for all the nominees except the Editorial Board. A vote by written ballot then elected S. R. Detwiler, R. S. Lillie, and H. J. Muller to the Editorial Board of the Journal of Morphology and Physiology.

The President read the appointment of the Nominating Committee for 1929: R. A. Budington, W. H. Longley, and Charles Packard.

### ELECTION OF NEW MEMBERS

The Executive Committee recommended and the Society elected 17 new active members, 4 associate members to active membership, 1 member reinstated to active membership, and 31 new associate members. The names of the new members are given in the following list. Associate members elected to active membership are indicated by an asterisk (*).

#### ACTIVE MEMBERS

ALTENBURG, EDGAR, A.M., Ph.D. (Columbia), Assistant Professor of Biology, *Rice Institute, Houston, Texas.*

*BISSONNETTE, THOMAS HUME, M.A. (Queens College), Ph.D. (University of Chicago), Professor of Biology and Head of the Department, *Trinity College, Hartford, Conn.*

BROWN, LELAND ARTHUR, S.B. (Denison), A.M. (Pittsburgh), Ph.D. (Harvard), Assistant Professor of Zoölogy, *State University of Iowa, Iowa City, Iowa.*

BUTLER, ELMER GRIMSHAW, A.B. (Syracuse), A.M., Ph.D. (Princeton), Assistant Professor of Biology, Princeton University, *Laboratory of Comparative Anatomy, Princeton University, Princeton, N. J.*

GARDINER, MARY S., A.B., M.A., Ph.D. (Bryn Mawr), Instructor in Biology, *Bryn Mawr College, Bryn Mawr, Pa.*

GLASER, RUDOLPH WILLIAM, A.B. (Michigan), Sc.D. (Harvard), Associate Member Rockefeller Institute, *Department of Animal Pathology, R.I.M.R., Princeton, N. J.*

GOWEN, JOHN WHITTEMORE, B.S. (Maine), M.S., Ph.D. (Columbia), Biologist, *Rockefeller Institute, Princeton, N. J.*

HARVEY, ETHEL BROWNE, Ph.D. (Columbia), Instructor, Washington Square College, New York University, *2 College Road, Princeton, N. J.*

*HERSH, AMOS HENRY, A.B. (Franklin and Marshall), A.M., Ph.D. (University of Illinois), Assistant Professor of Biology, *Department of Biology, Western Reserve University, Cleveland, Ohio.*

*HIBBARD, HOPE, A.B., A.M. (Missouri), Ph.D. (Bryn Mawr), Sc.D. (Sorbonne), Assistant Professor of Zoölogy, *Oberlin College, Oberlin, Ohio.*

*HOLMQUIST, ALBERT M., M.S., Ph.D. (University of Chicago), Professor of Biology, *St. Olaf College, Northfield, Minn.*

HOWLAND, RUTH B., Ph.B. (Syracuse), Ph.M., Ph.D. (Yale), Associate Professor of Biology, *Washington Square College, Washington Square, New York City.*

HUGHES-SCHRADER, SALLY, B.S. (Grinnell), Ph.D. (Columbia), Instructor in Biology, *Bryn Mawr College, Bryn Mawr, Pa.*

HUTCHINSON, GEORGE E., M.A. (Cantab.), Instructor in Biology, *Yale University, New Haven, Conn.*

KESSEL, JOHN FLENNIKEN, A.B. (Greenville), M.A., Ph.D. (California), Associate Professor of Zoölogy, *University of California at Los Angeles, Los Angeles, Calif.*

REDFIELD, ALFRED CLARENCE, B.S., Ph.D. (Harvard), Assistant Professor of Physiology, Harvard Medical School, *Brush Hill Road, Readville, Mass.*

SMITH, CHRISTIANNA, A.B. (Mt. Holyoke), A.M., Ph.D. (Cornell), Associate Professor of Zoölogy, *Mt. Holyoke College, South Hadley, Mass.*

TAYLOR, EDWARD HARRISON, A.B., M.S., Ph.D. (University of Kansas), Associate Professor of Zoölogy, *University of Kansas, Lawrence, Kan.*

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## MISCELLANEOUS BUSINESS

The Acting Secretary read the following letter from Dr. M. J. Greenman, Director of The Wistar Institute:

For some years past we have been making special concessions to members of the American Society of Zoölogists in supplying journals published by The Wistar Institute.

We wish to continue the same discounts on all our six journals according to the enclosed card, which is the same as in years past. We wish, however, to discontinue the sale of reprints at the special rate of $10.00 per 1000 pages.

We find that few members have availed themselves of this special rate, and it is far below cost in supplying them.

With these facts in view, it seems advisable to proceed as I have stated.

The Advance Abstract Sheets will be furnished without expense to members of the Society as in the past, and separate articles may be purchased according to the prices quoted on the Advance Abstract Sheets.

The Acting Secretary reported the receipt of the annual report of the Chairman of the Division of Biology and Agriculture of the National Research Council. The report was not read, owing to its length, but is abstracted and in part quoted below:

The first section of this report reviews the function of the Division. The second section states the urgent need for additional funds to permit the payment of expenses involved in the assembling of critical committees on such particular subjects as shall have previously met the approval of the various divisions of the Council. The third section lists the new activities of the Division. These cover the following topics: National Finance Committee on Botanical Nomenclature: Committee on the Effect of Irradiation upon Organisms; Fellowships in Forestry and Agriculture; Appropriate Language Requirement in the Different Science Fields; International Union of Population; Committee on Pharmacognosy and Pharmaceutical Botany; Experimental Animals and Plants for Investigators. The fourth section reviews the accomplishments of the Division during the year. The fifth section outlines the future of the Division.

A motion was made and carried that the Society continue the custom of defraying the expenses of its Secretary to the annual meeting by extending the same courtesy for this year to the Acting Secretary.

The President appointed a committee of two to draw up appropriate resolutions of thanks in connection with entertainment of the Society in New York. The Committee, consisting of L. B. Arey and E. L. Rice, presented the following resolutions:

*Resolved,* That in concluding a signally enjoyable and interesting meeting, the American Society of Zoölogists hereby wishes to tender an expression of thanks and appreciation for the hospitality extended to it by Columbia University and the American Museum of Natural History.

To the Local Committee, consisting of R. H. Bowen, G. K. Noble, H. W. Stunkard, and M. A. Bigelow, the gratitude of the Society is extended for the efficient manner in which accommodations were provided and the arrangements were made and carried out.

The meeting was then adjourned.

L. B. AREY, *Acting Secretary.*

# AMERICAN SOCIETY OF ZOÖLOGISTS

## *Officers for 1929*

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| *President* | Charles B. Davenport |
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| R. G. Harrison | 1929 |
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### *Representative of the Society in the Division of Biology and Agriculture of the National Research Council*

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|---|---|
| H. S. Jennings | 1932 |

Alternate, L. C. Dunn

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| | |
|---|---|
| C. A. Kofoid | 1932 |

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W. C. Allee    P. W. Whiting

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Ross G. Harrison    D. E. Minnich

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# MINUTES OF THE EIGHTH ANNUAL MEETING OF THE GENETICS SECTIONS OF THE AMERICAN SOCIETY OF ZOÖLOGISTS AND THE BOTANICAL SOCIETY OF AMERICA, HELD AT NEW YORK CITY, DECEMBER 27 TO 29, 1928

The 1928 meetings consisted of three sessions for the reading of papers on Thursday, Friday, and Saturday mornings, a joint meeting with "Geneticists Interested in Agriculture" on Thursday afternoon, a business meeting Friday noon, and informal laboratory demonstrations on Thursday and Friday afternoons. The attendance at the meetings averaged about 170. Prof. H. J. Muller, Chairman, presided at all sessions.

A total of 68 contributions were presented; 26 were read, 26 were given by demonstration, and 16 were read by title.

At the business meeting, the Chairman of the Interim Committee on the International Genetics Congress, Doctor Davenport, reported that, after a poll of American geneticists, it had been decided to accept the invitation of Cornell University and to hold the Congress in Ithaca between the 10th and 20th of August, 1932. The report of the Committee was unanimously accepted. On motion of Doctor Davenport, the following Committee was appointed by the Chair to be charged with the permanent organization of the Congress: T. H. Morgan, L. J. Cole, C. B. Davenport, E. M. East, R. A. Emerson, H. S. Jennings, and G. H. Shull.

The Committee on Organization, G. H. Shull, Chairman, reported that the results of a questionnaire inquiry indicated the existence of a widespread sentiment in favor of enlarging and broadening the scope of the genetics organization, but that agreement had not been reached as to the advisability of forming an independent genetics society. After some discussion, it was voted that the Committee as at present constituted (G. H. Shull, C. E. Allen, L. J. Cole, R. A. Emerson, H. S. Jennings, E. C. MacDowell, H. J. Muller, and O. E.

White) should be continued for one year, to report at the next annual meeting.

Prof. E. W. Sinnott was appointed to represent the Sections at the meetings of the Transportation Committee of the International Botanical Congress.

Prof. G. H. Shull was appointed the Sections' elector for representative to the National Research Council.

The following officers were elected:

| | |
|---|---|
| *Chairman* (for one year) | A. F. BLAKESLEE |
| *Secretary-Treasurer* (for three years) | P. W. WHITING |
| *Society Representative* (one year) | E. W. LINDSTROM |

L. C. DUNN, *Secretary,*
Columbia University, New York.

# THE RELATION OF PARITY, AGE, AND BODY WEIGHT TO THE NUMBER OF CORPORA LUTEA IN MICE

E. C. MacDOWELL, EZRA ALLEN, AND C. G. MacDOWELL
*Carnegie Institution of Washington, Department of Genetics, Cold Spring Harbor, Long Island, New York*

FIVE CHARTS

The number of corpora lutea per pregnancy rises with the parity (order of litter) when female mice are mated at four weeks old and bred continuously without nursing young. The rise is rapid for the early litters; in the later litters it slows down (MacDowell and Lord, '25 a).

The age of the mouse has less influence on the number of corpora lutea than has its previous reproductive history (MacDowell and Lord, '25 b). Within the same parity, as the age increases, the average number of corpora lutea remains practically the same. But for any given age the number of corpora lutea rises with the parity (fig. 1).

We now present entirely independent data on number of corpora lutea in which variation in parity is eliminated by using only first pregnancies; the females were not put with the males until twelve weeks old; body weights at conception are included. The corpora lutea in 678 single ovaries were counted by dissection immediately after a sudden mechanical death, while the blood still differentiated the most recent corpora lutea. To insure a high degree of accuracy in counts, 159 ovaries were sectioned. Since two corpora lutea are frequently flattened one against the other with an extremely thin wall separating them, the counting of corpora lutea from sections required not only minute histological study, but the counting of the number of sections per corpus.

THE ANATOMICAL RECORD, VOL. 41, NO. 3
FEBRUARY, 1929

The number of corpora lutea rises with the weight of the mouse (fig. 2), but the correlation is not linear. Above 24 grams, added weight is not associated with higher corpora

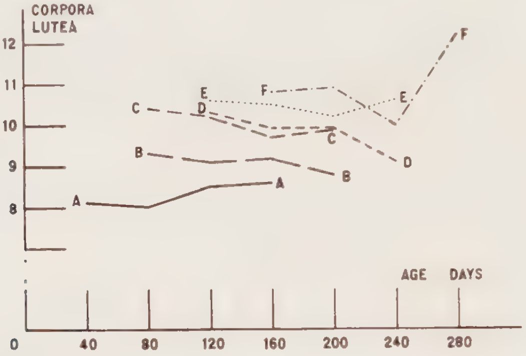

Figure 1

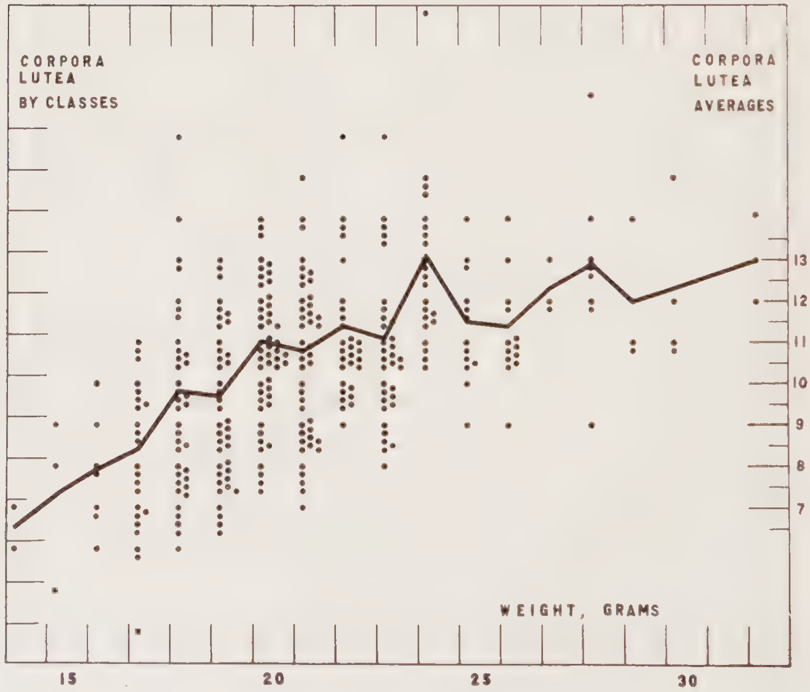

Figure 2

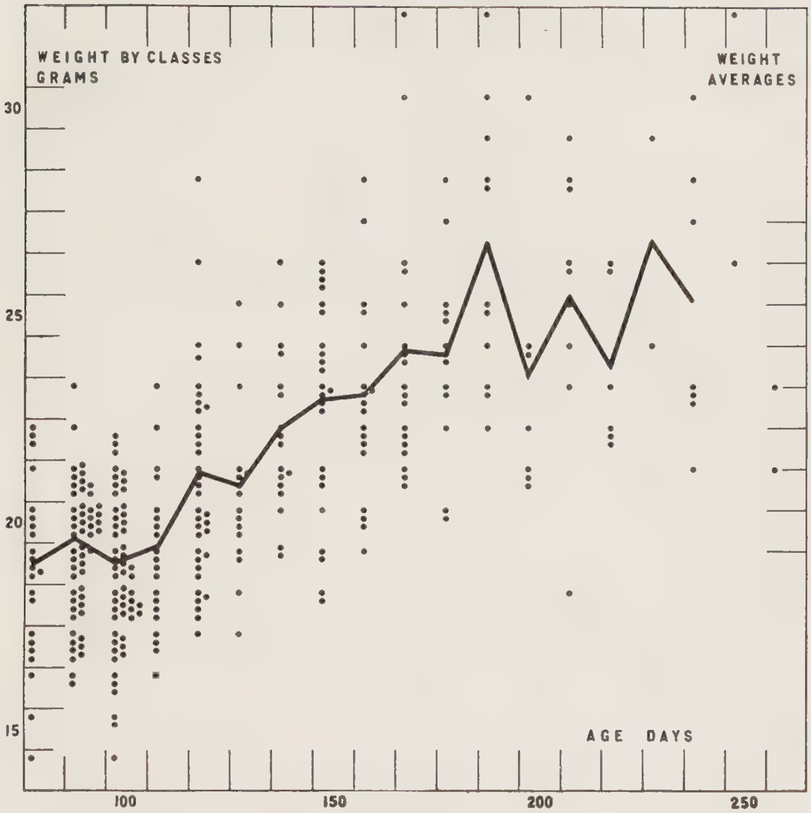

Figure 3

Fig. 1 Age vs. parity. Average number of corpora lutea of pregnancy classified by the age of the mother within the same parity. A, first litters, number of counts from left to right, 90, 101, 24, 8; B, second litters, number of counts, 114, 61, 15, 6; C, third litters, number of counts, 23, 84, 41, 10; D, fourth litters, number of counts, 46, 57, 29, 6; E, fifth litters, number of counts, 7, 35, 25, 8; F, sixth litters, number of counts, 14, 23, 12, 5; total counts, 844; points with fewer than five counts omitted. Corpora-lutea counts from surgical examination of ovaries in last week of pregnancy, technique, and data in detail given in MacDowell and Lord, '27. Only females put with older males when four weeks old are included.

Fig. 2 Distributions and averages of corpora-lutea counts according to conception weight of mouse; first litters only. Each dot represents a count of corpora lutea of pregnancy in a pair of ovaries examined immediately after a sudden mechanical death. Averages given by continuous line. Scale at left for corpora classes; at right, for averages. Figures given on scale are lower limits of the weight and corpora lutea classes.

Fig. 3 Distribution and averages of weight of female at conception, according to age. Same cases as in figures 2 and 4. Conventions as in figure 2.

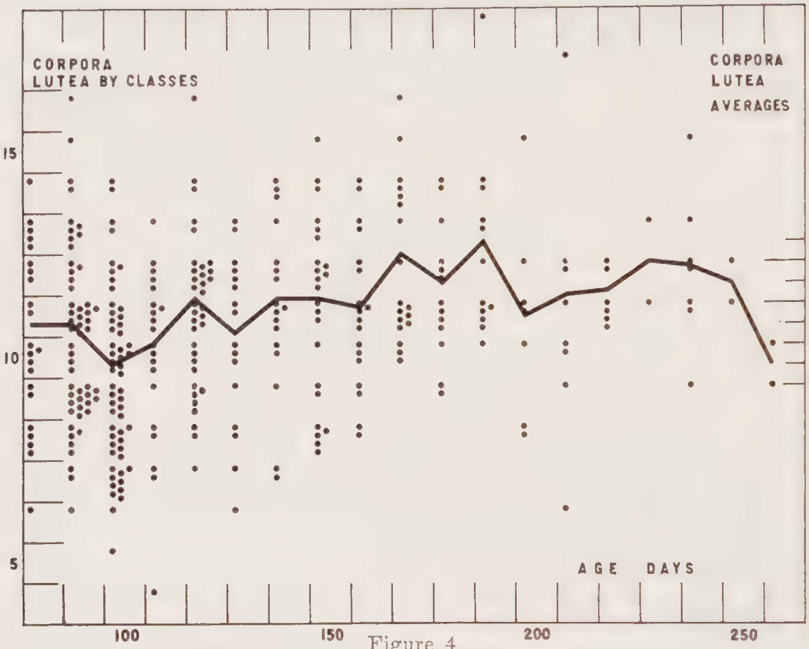

Figure 4

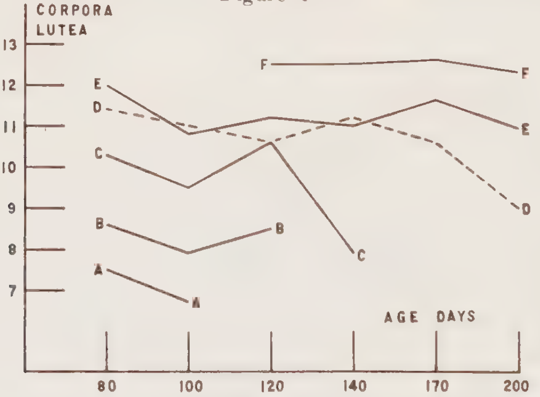

Figure 5

Fig. 4 Distribution and averages of corpora-lutea counts according to age of mouse at conception. Same cases as in figures 2 and 3; conventions as in figure 2.

Fig. 5 Weight vs. age in first pregnancies only. Average number of corpora lutea classified by age of mouse within the same conception weight class. A, conception weight 14–15 grams; B, 16–17 grams; C, 18–19 grams; D, 20–21 grams; E, 22–23 grams; F, 24–25 grams. Females not put with males until twelve weeks old. Lower limits of age classes given on the scale; above 140 days, these classes are increased from twenty to thirty days. With both parity and age held constant, the number of corpora lutea rises with the conception weight.

lutea counts; and as this limit is approached the relationship grows weaker. Although these data show that weight at conception is definitely correlated with age (fig. 3), the number of corpora lutea classified by age of mouse (fig. 4) show only a slight tendency toward a correlation. Within the same weight class, the average number of corpora lutea remains practically constant as the age increases, but for a given age the corpora-lutea averages regularly rise with the weight (fig. 5, table 1).

TABLE 1

*Averages of corpora lutea in first pregnancies classified by age and conception weight of female. Frequencies in brackets*

| GRAMS | DAYS | | | | | |
|---|---|---|---|---|---|---|
| | 80–99 | 100–119 | 120–139 | 140–169 | 170–199 | 200– |
| 14–15 | 7.5 (2) | 6.7 (3) | | | | |
| 16–17 | 8.6 (14) | 7.9 (14) | 8.5 (2) | | | |
| 18–19 | 10.3 (24) | 9.5 (32) | 10.6 (13) | 7.9 (7) | | 6.0 (1) |
| 20–21 | 11.4 (31) | 11.0 (21) | 10.6 (20) | 11.2 (13) | 10.6 (5) | 9.0 (5) |
| 22–23 | 12.0 (5) | 10.8 (5) | 11.2 (10) | 11.0 (19) | 11.6 (13) | 10.9 (10) |
| 24–25 | | | 12.5 (4) | 12.5 (12) | 12.6 (13) | 12.3 (6) |
| 26–27 | | | 11.0 (1) | 12.0 (6) | 12.3 (3) | 10.8 (6) |
| 28–29 | | | 12.0 (1) | 9.0 (1) | 12.8 (5) | 13.2 (5) |
| 30–31 | | | | | 11.0 (2) | 13.5 (2) |
| 32–33 | | | | | 13.5 (2) | 12.0 (1) |

Since Smith and Engle ('27) have demonstrated the powerful control exercised by the anterior pituitary substance over the number of ova in one ovulation in the mouse, and Hatai ('13), for the female rat, has shown a very high correlation between size of the pituitary gland and body weight, it seems probable that the positive correlation between body weight and the number of corpora lutea herein reported results from the correlation of each of these variables with the size of the anterior pituitary gland.

## SUMMARY

Counts of the corpora lutea of 1183 mouse pregnancies lead to the following conclusions: 1) Parity is more closely correlated with the number of corpora lutea of an ovulation than is the age of the mouse; 2) When parity is eliminated by using only first pregnancies, weight at conception is more highly correlated with the number of corpora lutea than is the age of the mouse.

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# CILIATED EPITHELIUM AND MUCUS-SECRETING CELLS IN THE HUMAN HYPOPHYSIS[1]

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ONE HELIOTYPE PLATE (SIX FIGURES)

My interest in the ciliated epithelium of the human hypophysis was first aroused in 1916 by Bryant's paper entitled, "Sensory elements in human cerebral hypophysis." During the last few years we have been looking for these ciliated cells. Having failed to see anything that could be interpreted as cilia with certainty, the author (Rasmussen, '28 a) felt justified in calling attention to this discrepancy, which was done in the following words: "Strange to say such ciliated cells have never been seen in any of the series examined in this laboratory now numbering well over 200." Soon after this was in press, a series showed up in which such cells were clearly evident both in the residual lumen and in a small cyst in the posterior and lateral portion of the anterior lobe. This hypophysis is from a twenty-eight-year-old man who died after a decompression operation to relieve pressure caused by a glioma 5 cm. in diameter situated in the left occipital lobe.

The hypophysis was removed six hours after death and fixed in formalin. The gland weighed 554 mgm. (without capsule and stalk), and all the subdivisions of the organ are near the average or well within the range of normality as established by an accurate volumetric analysis of 111 normal male adult hypophyses from sudden accidental death (Rasmussen, '28 b).

Since then another case (seventy-one-year-old man who was killed by fracture of the skull and broken neck as a result

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[1] Aided by a grant from the research fund of the Graduate School, University of Minnesota.

of falling down an elevator shaft) has been found which also contains ciliated cells in the residual lumen. This hypophysis was removed three hours after death and similarly fixed in formalin. It weighed 511 mgm. (without stalk and capsule) and is normal in all other respects.

On the assumption that the search in the past had not been sufficiently careful or that the cilia disappear rather rapidly after death, one hundred normal male adult hypophyses obtained twelve hours or less (some as early as one hour) after death were reexamined under the oil-immersion lens, but only the two cases mentioned above could be said with certainty to possess ciliated cells. Peremeschko ('67) remarks that they are difficult to make out, except in fresh material. In several other specimens striations in the colloid or in the shrinkage space next to the epithelium lining the residual lumen and related cavities were seen that suggested cilia, but careful examination under oil-immersion lens failed to show definitely that they were actual cilia.

In contrast to this is the statement of Bryant ('16) that such cells were found in all the freshly hardened human hypophyses which he examined, except those in which the parenchyma had been almost completely replaced by connective tissue, although he encountered them in some hypophyses which had undergone very extensive pathological changes. They are described as occurring in maculae, mostly on the posterior wall of the residual lumen and sometimes apparently on the anterior wall, that is, lining the posterior part of the anterior lobe. Interspersed between some of the ciliated cells he describes a few bipolar non-ciliated cells with peripherally placed nuclei. Judging from his illustration, these are very similar to the degenerating cells described by Vanderburgh ('17) in the guinea-pig. In addition, he shows a layer of round cells between the ciliated elements and the basement membrane.

In searching through the literature one finds only occasionally an author that makes note of the presence of ciliated cells in the residual lumen of the human hypophysis—Peremeschko ('67), Joris ('07), Thaon ('07), Dialti ('10), Gierke ('21), Erdheim ('26), Kiyono ('26), and Guizzetti ('26) are specific examples. Of several available recent text-books on histology, only the one by Lewis and Stöhr (third edition by Bremer, 1927) seems to mention this fact; however, the entire list has not been checked. Boyce and Beadles ('93), after examination of more than a hundred human glands and some from monkeys, state definitely that they never saw ciliated epithelium either in human or monkey material. In cysts (in or near pars intermedia) which are presumably, in many cases at least, outgrowths from the residual lumen (a subject discussed in some detail in a previous paper (Rasmussen, '28 a —see also Guizzetti, '26)) such cells have been described by at least Henle ('65), Müller ('71), Krause ('76), Launois ('03), Tello ('12), Kiyono ('24), Erdheim ('26), Guizzetti ('26), and Bailey ('28). It is difficult to get a reasonably complete list of references, because a subject like this is usually only an incidental part of a larger topic, and hence difficult to pick out of bibliographies. On the whole, however, it would seem that if such cells were regularly present they would have been noticed more frequently by others. Guizzetti ('26), for example, found cilia in the residual lumen in only three out of fifty-four specimens. These were, respectively, ten, twenty-seven, and thirty-six years of age. Kiyono ('26) found one in fifty cases, and that was premature.

It appears worth while to see how extensively ciliated cells occur in the hypophysis of animals generally. A brief summary of the comparative aspect follows.

A capillary duct, lined with ciliated epithelium and establishing a connection between the buccal cavity and the neural canal during larval stages, was considered by Andriezen ('94) to be a part of the hypophysis of amphioxus and ammocetes. The homology of the hypophyseal complex in these low forms is, however, uncertain. De Beer ('26) concludes that in the adult amphioxus the hypophysis is represented by the ciliated organ of Müller (a development from the walls of the preoral pit), whose function probably is to create a current of water.

Later, phylogenetically, according to this author, it sank beneath the surface, but remained in communication with the exterior by means of a duct. Still later it became closely associated with the infundibulum of the brain, and its communication with the surface was obliterated. If this is the true phylogenetic history, the occurrence of ciliated cells in the residual lumen might not be unexpected here and there in later forms.

In the chromophilic part of the hypophysis of the domestic fowl, Collin ('26) found small cysts, just visible to the naked eye, which were lined to a variable extent with ciliated columnar cells, mucus-secreting cells, and intermediate forms. Cuboidal and even squamous cells were also found. In places the epithelium had desquamated. The contents of these cysts reacted like mucin with mucicarmin, and not like colloid.

In still higher animals Trautmann ('09) thought he saw some cilia-lined cavities in the hypophysis of the pig, but was not certain. In a large number of other domestic animals he failed to find any suggestion of cilia. However, Lothringer ('86), Rogowitsch ('89), and Stieda ('90) state that ciliated cells occur in some of the vesicles of pars intermedia of the rabbit. In cattle Dostoiewsky ('86) found cavities in the anterior lobe which were lined with ciliated epithelium.

It is in the guinea-pig, according to the report of Vanderburgh ('17), that ciliated epithelium seems to be most regularly present both in the residual lumen and in near-by vesicles or cysts. In the cleft it was present in all animals examined, usually in its posterior end, but did not completely line the cavity, being found only on the side next to the anterior lobe and never on the side next to pars intermedia. Ciliated cells were less often present in the vesicles. Some ciliated cysts were seen practically outside of the gland in the anterior region near the original attachment of Rathke's pouch. These, he thinks, probably originated from persisting portions of the stalk.

The ciliated cells in the two human cases reported here (figs. 1 to 5) were irregularly distributed. They were mostly found on the side of the residual lumen next to the anterior lobe. Only a few were found on the opposite wall. They were also more abundant in the pole farthest away from the infundibular attachment. The ciliated cells in the small cyst of the anterior lobe, already referred to (fig. 2), appear to be essentially of the same type as those in the residual lumen (fig. 1).

Nothing resembling the maculae of the inner ear was ever seen. In places the basement membrane is increased to a well-developed stratum of connective tissue, as shown in figure 1, but not enough to form localized elevations. There is often a layer of more nearly round cells interposed between this connective-tissue membrane and the columnar cell, making the epithelium two-layered—rarely more than that.

The reaction of the typical columnar ciliated cells is not specific. Among them is an occasional eosinophil, such as are regularly present in the anterior lobe proper. Such a cell is illustrated in figure 2, where it is represented by the non-ciliated and more darkly shaded cell in the epithelial wall at the left of the drawing.

Both in the residual lumen and in the cyst the ciliated cells are usually 15 μ in length. A few are somewhat shorter. The cilia are in the neighborhood of 5 μ in length. The free border of the cell is sharply defined, and immediately beneath are the usual basal bodies. The general structure of the cell body and nucleus is illustrated in figures 1 to 5. As shown, the cytoplasm is more or less granular or vacuolated, depending upon the technique. Here and there individual cells or groups of cells (figs. 3, 4, 5) are greatly distended with a substance that gives a strong mucin reaction. Figure 4 shows a section stained with mucicarmin. The dilated end of the greatly swollen cells is stained intensely red. As illustrated, especially in figure 3, some of these cells present the general features of a typical goblet cell, the nucleus being highly chromatic and displaced to the basal end, which is usually greatly narrowed. Some of these goblet cells attain to a length of 30 μ. Intermediate stages between the long columnar ciliated cells and these enlarged non-ciliated cells are in evidence.

The contents of the lumen, adjacent and frequently adherent to these mucoid cells, also show a tendency toward the mucin reaction. In no case are either ciliated or mucoid cells closely associated with typical colloid. There are masses of colloid in the residual lumen, but between these and the cells under discussion there is a substance that seems to be more fluid and to contain degenerating cells and other granular material.

The significance of these ciliated cells is not clear. The conclusion of Bryant ('16) that they represent a sensory epithelium does not seem to be warranted. His illustration shows among taller and more cylindrical cells many large clear dilated cells which greatly resemble the 'goblet' type. The nucleus is displaced to the base and the distended part of the cell is distinctly different from the cytoplasm of the more columnar cells.

The very narrow elongated cells, with nuclei displaced peripherally, which Bryant designates 'bipolar cells,' are apparently either the result of pressure produced by the enlarging clear ('goblet') cells, such as is shown particularly in figure 5, or are degenerating cells. In fact, none of the cells have even a general morphological resemblance to the sensory cells of the inner ear.

While Tello ('12), who has probably done the most reliable work on the finer relations of nerve fibers to the epithelial cells in the region of pars intermedia of the human hypophysis, shows nerve fibers ramifying among the basal ends of the cells lining the residual lumen and associated vesicles, and interprets his findings as suggesting a differentiated sensory mechanism of some unknown function, no special relations of nerve fibers to the ciliated cells have been demonstrated as far as the writer can ascertain. It might be added that Gentès ('03) was also rather struck with the similarity between pars intermedia of the cat and dog and the sensory epithelium of the ear and olfactory membrane.

Ciliated epithelium is found pathologically in a great variety of places (see, for example, Zahn, '96), usually lining cysts of congenital origin; and the hypophysis seems to be no exception (Beck, '83; Fulstow, '28). It may be that the ciliated cells here described in essentially normal hypophyses should be considered pathological. The contents of the residual lumen in both cases suggest that the remains of Rathke's pouch have become cystic, using the term 'cystic' in the narrower sense.

The question of metaplasia must, of course, be considered. The cells immediately about the lumen of the glandular part of the hypophysis assume a variety of forms during development, as has been discussed most recently by Guizzetti ('26), Lewis and Lee ('27), and the writer (Rasmussen, '28 a). They may become squamous, cuboidal (polygonal), or columnar in shape and posteriorly form either a single layer or a stratified sheet of irregular thickness. The polygonal and strongly basophilic cells that frequently invade the posterior lobe may also be derived from pars intermedia, as is maintained particularly by Guizzetti ('27), though some consider that they have migrated in from the anterior lobe.

During early childhood tubular gland-like outgrowths from pars intermedia into the posterior lobe are common. In the twenty-eight-year-old hypophysis, here described as containing ciliated cells both in the residual lumen and in a separate cyst, there is also a persistence of these glands, as shown in figure 6. The walls of these branched tubular glands consist of a single layer of columnar cells about 15 μ in height. The diameter of the tubules varies from around 30 μ to about 50 μ. They are relatively rare in adults, this being the only specimen found in fully 200 adult hypophyses that we have cut serially. Guizzetti ('26) claims that the substance which is secreted by some of the evaginations from the pars intermedia gives the microchemical reaction of mucin. While in this adult only a few of the cells of these glands contained granules that gave a mucin reaction, it is not inconceivable that pars intermedia may give origin to mucous cells.

It is also possible that the ciliated cells of the hypophysis are abnormal migrations of embryonal elements from the nasopharynx, where ciliated and mucus-secreting cells are normally present.

A less likely source of the cells undergoing mucoid degeneration is from neuroglia cells that have grown in from the adjacent posterior lobe. It is claimed (Grynfeltt, '26) that neuroglia, and particularly oligodendroglia, may undergo mucous degeneration.

In this connection it may be recalled that Luschka ('60) thought he found in a young human specimen remains of the infundibular cavity in the form of ciliated epithelium in the posterior lobe. It is possible that what he saw was a diverticulum from pars intermedia, so that an ependymal origin need not be taken seriously.

To summarize, it appears that tall columnar cells with numerous delicate cilia occasionally occur in the epithelium lining the residual lumen and cysts in the region of pars intermedia of the human hypophyses that otherwise are essentially normal. Some of these cells apparently secrete a mucus-like material or undergo mucoid degeneration. There is no significant evidence that they are sensory elements. Their origin is uncertain, but probably should be looked upon as unusual differentiations of hypophyseal tissues or as migrations of nasopharyngeal elements during early stages of development. Branched tubular glands, so common in the hypophysis of young children, may be present in adults. The nature of the substance which these glands secrete is uncertain.

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## PLATE 1

### EXPLANATION OF FIGURES

1 Camera-lucida drawing of a portion of the anterior wall of the cystic residual lumen of the hypophysis of a twenty-eight-year-old man, showing the general characteristics of the ciliated epithelium. Six hours postmortem. Formalin fixation. 5 μ. Hematoxylin and eosin.

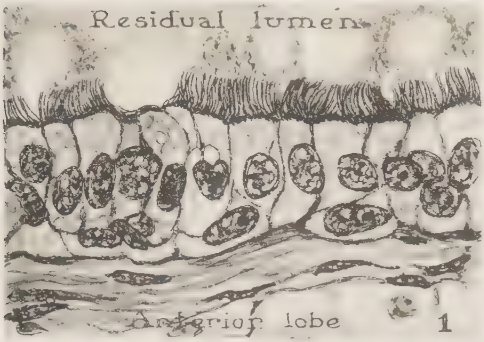
2 Camera-lucida drawing of the ciliated epithelium lining a small cyst in the posterior part of the anterior lobe of the same hypophysis as in figure 1. The dark non-ciliated cell to the left, among the ciliated ones, is an eosinophilic cell of the type regularly found in the anterior lobe. The very dark cell to the right and below the epithelium of the cyst is a characteristic basophil. Same technique as in figure 1.

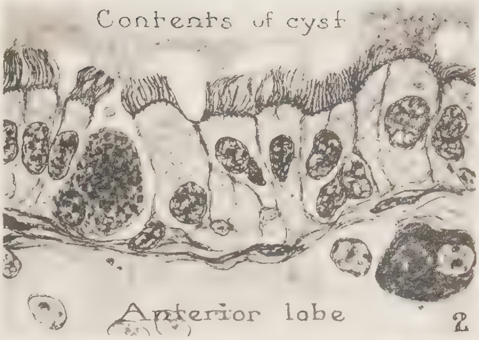
3 Camera-lucida drawing of goblet-like cells discharging their mucous contents into the residual lumen. They are apparently transformed ciliated cells. From a seventy-one-year-old man. Three hours postmortem. Formalin fixation. 5 μ. Iron hematoxylin.

4 Camera-lucida drawing of a section adjacent to the one shown in figure 3 stained with mucicarmin. The intensely red color reaction of the contents of the 'goblet' cells is indicated by dark shading.

5 Camera-lucida drawing, showing mucous degeneration in some of the lining cells of the small cyst in the posterior portion of the anterior lobe. Same specimen as in figure 2. 5 μ. Mallory's connective-tissue stain.

6 Camera-lucida drawing of a group of tubular glands in the posterior lobe (adjacent to pars intermedia) of an adult man (same specimen as in figs. 1, 2, and 5). 5 μ. Hematoxylin and eosin.


Residual lumen
Anterior lobe
1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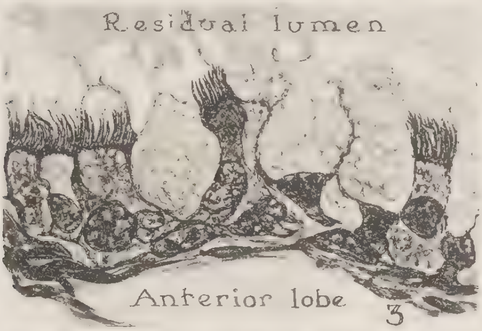
Anterior lobe
2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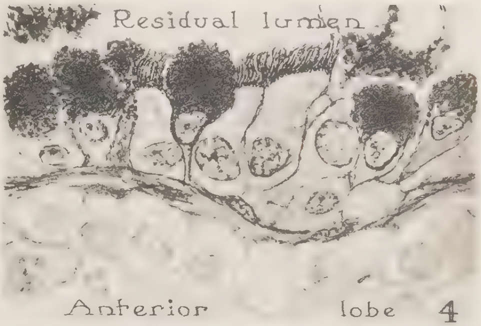
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Anterior lobe
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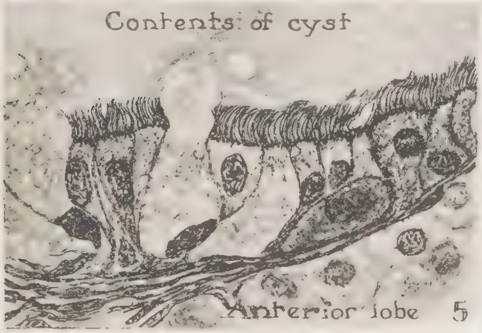
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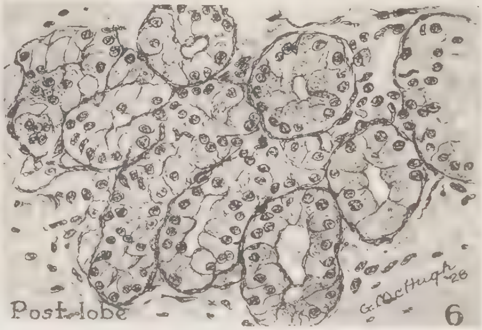
Anterior lobe
5


Post. lobe
G. McHugh '28
6

# CONTRIBUTION TO THE NERVE SUPPLY OF THE DIAPHRAGM[1]

F. KISS AND HARRY C. BALLON
*Budapest and Montreal*

FIVE FIGURES

The surgery of the phrenic nerve and of the diaphragm occupies at the present time a position of considerable interest. The extensive literature, contributions along clinical and morphological lines which have become available during the past few years bear witness to this fact. Nevertheless, in spite of this vast literature, anatomists and clinicians still find that important questions concerning the innervation of the diaphragm have been replied to differently by various authors or have even remained unanswered. Statements which contradict well-known morphological and physiological facts are to be found even in the more recent discourses and have apparently been accepted by many.

We do not wish to present any new treatise on the diaphragm as a whole. W. Felix(10) and K. Hitzenberger(14) have already done this in most able works. Our purpose was rather to examine carefully the phrenic nerve, nervus diaphragmaticus of the splanchnic plexus, the intercostal nerves, and the vagus nerve, both macroscopically and microscopically, and in the light of very recent experiences to endeavor to correlate our observations with these new experiences wherever possible.

All our investigations were carried out upon human material.

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## NERVUS PHRENICUS

As a result of recent investigations, chiefly phrenicotomies, there exists no longer any doubt that the phrenic nerve is the most important motor nerve supply to the diaphragm. According to Luschka(18), Pansini(19), Cavalié(7), Henle(13), Schwalbe(23), Timofejew(24), Ellenberger(9), Baum, Felix, and others, the phrenic nerve does not innervate the entire diaphragm. These authors believe that the diaphragm is innervated in its midportion by the sympathetic and the lateral segments on both sides by the XIIth intercostal nerve. Another group of equally well-known investigators, among whom may be mentioned Bertelli(2), Ramström(20), Russel (21), Fuchs(11), Eisler(8), Schlaepfer(22), and Lobmayer (17), maintain that the phrenic nerve is the only motor nerve to the diaphragm. Clinical observations, such as referred shoulder pain, speak in favor of the phrenic nerve also containing sensory fibers.

The many anastomoses between the sympathetic, the ganglion stellatum, and its neighboring branches have also been described by Felix, K. Yano(25), and others. Luschka was the first to note the anastomosis between the phrenic nerve and the coeliac plexus by way of the phrenic plexus and ganglion phrenicum.

The anastomosis of the phrenic nerve with the sympathetic has been wrongly interpreted by some authors who have advanced theories which cannot be accepted. W. Felix has stated the following:

> 1. The phrenic nerve alone supplies the central parts of the serous covering of the diaphragm with sensory fibers. The nature of its sensibility lies not in its own spinal fibers but in the fact that the phrenic nerve contains sympathetic fibers which conduct centripetally.
>
> 2. The importance of the connection between the phrenic nerve and the sympathetic lies in that the terminations of the phrenic which end in the serosa are not spinal endings of the phrenic but endings of the sympathetic cable which run in the phrenic.
>
> 3. The diaphragm receives its motor nerve supply from the phrenic nerve, the XIIth intercostal nerve and probably from the sympathetic nerve. The phrenic nerve supplies the main portion of the dia-

phragm; the XIIth intercostal the origin of the muscle from the twelfth rib, and the sympathetic and phrenic nerves conjointly the lumbar segment of the diaphragm.

Hitzenberger has transferred these ideas of Felix into his monograph and has apparently accepted them.

Japanese authors Shimbo and Aoyagi(1) apply another significance to the sympathetic fibers in the phrenic nerve. Their investigations have shown that the cutting through of the spinal roots leads to an inactivity atrophy of the diaphragm, but that the cutting of the sympathetic fibers leads to an actual degeneration of the musculature.

After we had satisfied ourselves that the phrenic trunk anastomoses in the upper thorax with the sympathetic by many fine strands, we directed our attention to the microscopic appearance of the phrenic nerve. In its microscopic picture we found three well-differentiated forms of nerve fibers (fig. 1).

In every segment the majority of the phrenic fibers are medullated. Only here and there do we find small groups of non-medullated sympathetic fibers scattered among the former (fig. 1, *a*). Among the medullated fibers we could differentiate two forms: *a*) thick richly myelinated fibers, 6 to 15 μ in diameter (fig. 1, *c*); *b*) thin medullated fibers, 2 to 4 μ in diameter (fig. 1, *b*). The thickness of the myelin covering in the former measured 2 to 4 μ and in the latter 1 μ or less. The majority of the fibers, however, are of the thick medullated type, while the thin ones are only scattered and occur singly. The relative count of the non-medullated fibers is not greater in the phrenic nerve than in the other spinal nerves, as, for example, the intercostal nerves. From the investigations of K. Yano we know that the count of the non-medullated fibers below the anastomosis of the phrenic with the middle and lower cervical ganglion is increased, in that the anastomosing branches consist mostly of non-medullated fibers. The majority of the fibers even here belong to the group of the large medullated fibers.

We are in a position to explain the function of the various fibers in a more exact manner than has been found in the literature up to the present time. As a result of systematic investigations, F. Kiss and P. von Mihálik(16) were able to find a connection between the various peripheral nerve fibers and their function. These investigators have shown that the motor fibers have thick medullated sheaths, the sensory fibers thin medullated sheaths, while the sympathetic fibers are non-medullated. The thick fibers in the phrenic nerve are therefore motor, the thin fibers are sensory. The non-medullated fibers arise from the sympathetic trunk.

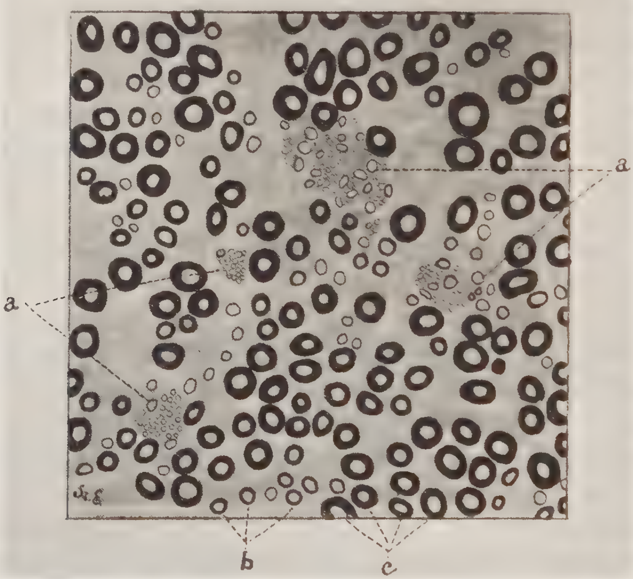

Fig. 1 Drawing showing the microscopic appearance of the phrenic nerve on transverse section, stained with osmic acid. *a,a*, groups of sympathetic fibers; *b*, fibers with thin medullary sheaths; *c*, fibers with thick medullary sheaths.

The so-called preganglionic autonomic and the parasympathetic fibers have likewise thin medullary sheaths and they cannot be differentiated from the sensory fibers. Such autonomic fibers, however, play no significant rôle in the phrenic nerve. The motor spinal nerves and the cerebral nerves have the same kind of medullated fibers as the phrenic nerve, and the microscopic picture of the phrenic nerve coincides with that of the usual motor nerve. So, too, for example, such muscles as the eye muscles which, according to previous anatomical findings, were considered as being innervated by a so-called brain nerve, also have sensibility. This in spite of the deficiency or absence of an anatomical sensory branch, as, for instance, from the trigeminus. Kiss and Mihálik found the sensory nerves of such muscles. They are present as thin medullated fibers in motor nerves.

There is, therefore, no morphological basis for the belief in a double innervation of voluntary muscle. Kiss and Mihálik have shown that the so-called accessory sympathetic fibers of Boeke, Dusser de Barenne, Kultschitzky, Hunter (15), and others are apparently thin medullated sensory fibers. The scanty sympathetic fibers enter the muscle with the blood vessels. Our investigations concerning the innervation of the diaphragm confirm these findings. The thick medullated fibers of the phrenic nerve are therefore motor, while the thin medullated fibers are sensory. Both have a spinal origin. In this way the morphological basis for the sensibility of the phrenic nerve is explained. The non-medullated fibers of the phrenic which are led to the sympathetic either through the accessory or 'neben' phrenicus or through direct anastomosis are of a purely sympathetic nature. They have nothing to do with the motor or sensory innervation of the diaphragm according to the manner described by Felix.

The non-medullated fibers, as we have already noted, do not occur in any greater number in the phrenic nerve than they do in most of the spinal nerves. They are carried to them by the rami communicantes. We have also no reason to believe that the fibers in the phrenic nerve have a function which differs from that of ordinary spinal nerves. The most familiar function of the sympathetic nerves in striped muscle is a vasomotor one. Very noteworthy are the observations of Shimbo and Aoyagi. They report that the cutting of the phrenic nerve leads to an 'inactivity' atrophy; that section of the sympathetic fibers leads to a degeneration of the musculature of the diaphragm. From these observations it is to be assumed that the sympathetic fibers also perform a trophic function.

The anastomosis of the phrenic nerve with the coeliac plexus will be dealt with later.

Such conclusions as those (1, 2, 3) which we have quoted from the work of Felix compel us to state clearly that the sympathetic conducts only centrifugally; that its function is exclusively motor, secretory, perhaps also trophic. All centripetal fibers which occur in the sympathetic or sympathetic plexus are of spinal origin and reach the sympathetic through the rami communicantes. We have up to the present time no reason to assume that the sympathetic has any action on voluntary striped muscles. We found no smooth muscle on microscopic examination of the diaphragm. Even the sphincter-like portion around the oesophagus consists only of striped bundles.

## NERVI INTERCOSTALES

Some investigators suggest that intercostal nerves take part in the motor innervation of the diaphragm. Felix maintains, in fact, that that portion of the diaphragm which lies under the twelfth rib is innervated solely by a branch of the XIIth intercostal nerve (see figures, Felix, p. 317, and Hitzenberger, p. 121). Eisler(8), v. Gössnitz(12), and Ramström have, on the basis of their own careful anatomical studies, denied the motor innervation of the diaphragm by the intercostal nerves. Clinical observations also speak in favor of the latter view.

Felix founds his statements and conclusions upon the examination of an embryo of the sixth month by serial section. Only in this single specimen did he observe that a branch of the XIIth intercostal nerve entered the diaphragm. This branch, writes Felix, ran at first cranially, then in a ventral direction on the prong of the twelfth rib. At first it accompanies a small artery. It separates itself from the artery, however, shortly before it enters the diaphragm, which it does by itself. Here it enters into a ventrolateral direction. He states: "I emphasize the fact that the nerve runs only for a short distance with the artery and then enters the diaphragm unaccompanied." Felix states further that, to determine if it were really a motor branch, he followed the branches of the phrenic nerve in the musculature of the diaphragm as far distal as possible. This was in an endeavor to recognize the endings of the branches. But he remarks that he could not demonstrate anatomical end plates or direct entrance of the nerve into muscle fibers. In an embryo of the sixth month one cannot with absolute certainty designate or call a nerve ending motor. One can only suggest such a probability or possibility. In this connection probability or possibility concerning the motor function of the XIIth intercostal nerve is really all that one is permitted to suggest. The remaining intercostal nerves have no branches which would suggest a motor innervation of the diaphragm (Felix, pp. 307, 308).

We examined the intercostal nerves in several adults and children. By careful preparation we found, as did Ramström (20) and others, fine, hardly preparable, lateral branches (fig. 2, *d*,*d*). These branches ran from every intercostal nerve to the points of origin of the diaphragm. In no specimen did we observe that the XIIth intercostal nerve gave off a larger branch than did the other intercostal nerves. The microscopic examination of these fine branches with osmium showed that about half of the fibers were of the thin type. The other half had thick medullated sheaths. Since Kiss and Mihálik (loc.cit.) showed that some of the thick richly myelinated fibers are to be found under the thin fibers in the spinal roots, also in many sensory fibers that are probably centripetal, the exact function of these latter fibers being unknown, we are not in a position to state whether the fibers which we found here are sensory or motor. After reviewing the experiences of Felix as well as our own investigations, we do not consider that there is a motor innervation of the diaphragm by the intercostal nerves. The complete paralysis of the diaphragm after an exact phrenicotomy also speaks in favor of those who deny the motor innervation of the diaphragm by the intercostal nerves.

In operative cases Capps(6) and Coleman(5) introduced a wire into the pleural cavity and irritated the pleura diaphragmatica. They found that irritation of the central portion of the diaphragm produced the same sort of pain in the shoulder and neck as does stimulation or cutting of the phrenic trunk. When they touched the anterior, lateral, and posterior margins over an area 3 to 5 cm., it led to pain which was referred to the lower half of the thorax: in other words, to pain which was felt over the region supplied by the lower five to six intercostal nerves. Stimulation of the peritoneal surface of the diaphragm gave similar results.

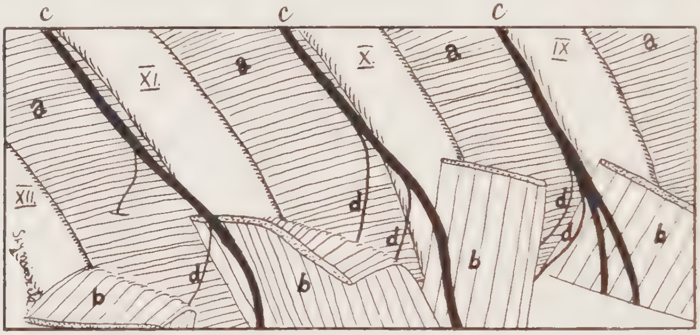

Fig. 2 Drawing showing the distribution of the intercostal nerves with their branches to the diaphragm in man. *IX–XII*, IX to XII ribs; *a,a*, intercostal muscles; *b,b*, points of origin of the diaphragm; *c,c*, intercostal nerves; *d,d*, branches of the intercostal nerves to the diaphragm.

All these anatomical and clinical findings lead one to conclude that the margins of the diaphragm and its pleural and sensory surfaces are innervated with sensory fibers from the lower five to six intercostal nerves.

## PLEXUS DIAPHRAGMATICUS

This plexus is but a small portion of the large widespread splanchnic plexus. Luschka described the anastomosis between the phrenic nerve and the splanchnic plexus and the ganglion phrenicum as early as 1853. We present in figures 3 and 4 original plates from our preparations. We have noted that the position of the so-called phrenic ganglion is variable. The branches of the small diaphragmatic plexus unite with the suprarenal plexus by several anastomosing fibers which apparently follow the phrenic artery. The branches of this plexus end in the substance of the diaphragm. One of its branches can, as Luschka already noted, anastomose with a terminal branch of the phrenic nerve.

We also examined the branches of the diaphragmatic plexus microscopically (fig. 5). The greater number of the fibers are non-medullated, sympathetic. Among them run solitary fibers which have thin medullated sheaths (fig. 5, *b*). We also found richly myelinated thick fibers (fig. 5, *c*). From the investigations of Kiss and Mihálik we know that all the medullated fibers in the sympathetic come from the spinal nerves that run through the rami communicantes, through the splanchnics, to the sympathetic and from there to the inner organs.

The medullated fibers which join the sympathetic are of two forms: *a*) ordinary sensory fibers; *b*) preganglionic fibers. The ordinary sensory fibers *a* course, for example, in the abdominal cavity by way of the splanchnic plexus to the abdominal organs. The fibers *b* run to the root ganglia and then to the so-called prevertebral, coeliac, renal, and phrenic ganglia, etc. Both forms of fibers have similar thin medullary sheaths (fig. 5, *b*). We also found thick medullated fibers in the peripheral branches of the coeliac plexus as in figure 5, *c*; in other words, fibers which resemble the motor fibers to voluntary muscle. We do not know of any voluntary motor innervation in the abdominal cavity. We must therefore assume for the present that these thick richly myelinated fibers are the same thick fibers that escaped

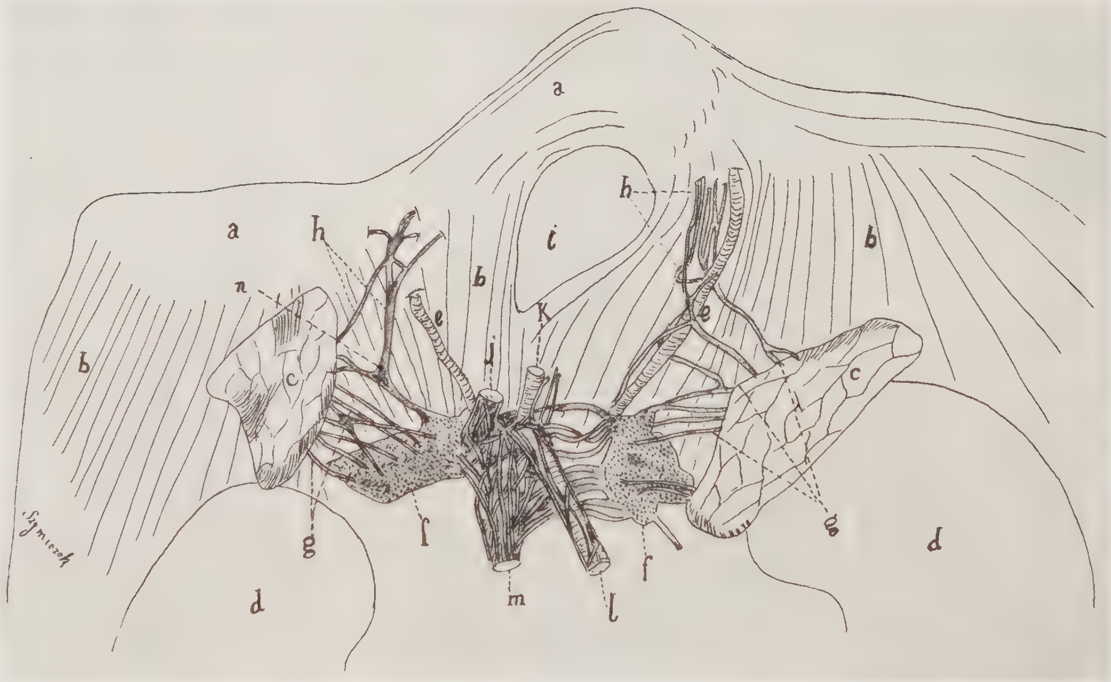

Fig. 3 Drawing showing the coeliac ganglia with the coeliac plexus in man. *a,a,* centrum tendineum of the diaphragm; *b,b,* diaphragm; *c,c,* adrenal gland; *d,d,* kidney; *e,e,* inferior phrenic artery; *f,f,* coeliac ganglion; *g,g,* suprarenal plexus; *h,h,* diaphragmatic plexus; *i,* hiatus oesophageus; *j,* hepatic artery and hepatic plexus; *k,* left gastric artery and plexus; *l,* splenic artery and plexus; *m,* superior mesenteric artery and superior mesenteric plexus; *n,* phrenic ganglion.

between the thin fibers of the posterior spinal roots. A more exact function of these fibers is up to the present time unknown.

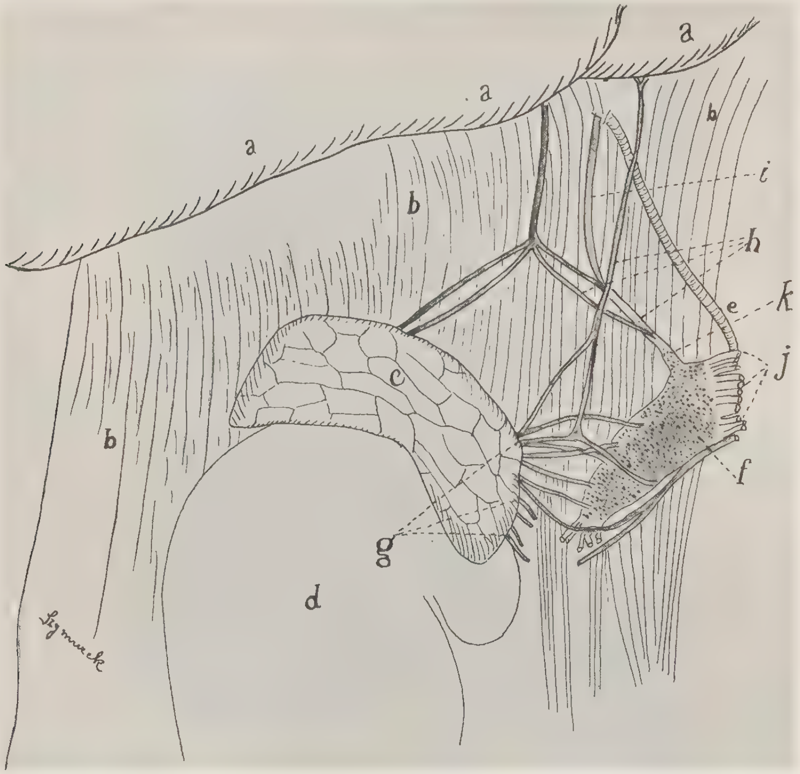

Fig. 4 Drawing showing the coeliac ganglion and the diaphragmatic plexus on the right side. *a,a,* liver; *b,b,* diaphragm; *c,* adrenal; *d,* kidney; *e,* inferior phrenic artery; *f,* coeliac ganglion; *g,* suprarenal plexus; *h,* diaphragmatic plexus; *i,* largest branch which runs from the diaphragmatic plexus to the diaphragm; *j,* branch from the coeliac ganglion to the plexus and to the opposite side; *k,* ganglion phrenicum.

The vast majority of the fibers in the diaphragmatic plexus are therefore of a sympathetic nature. As we pointed out above, we found no smooth musculature in the diaphragm on microscopic examination. The action of the sympathetic nerve fibers found in the diaphragm is either vasomotor or perhaps, as mentioned by Shimbo and Aoyagi, trophic. We see no reason to assume that the coeliac plexus or that the sympathetic takes any part in the innervation of the musculature of the diaphragm. The theory of a spinal and sympathetic, double innervation of striped muscle in the sense of Boeke(3), Dusser de Barenne(4), and others is, according to the experience of Kiss and Mihálik and from our own, no longer to be held. The thin medullated fibers of the plexus to the diaphragm are partly preganglionic to the ganglion phrenicum and to the scattered ganglion cells in the substance of the diaphragm. Some of them can be sensory fibers to the lumbar portion of the diaphragm.

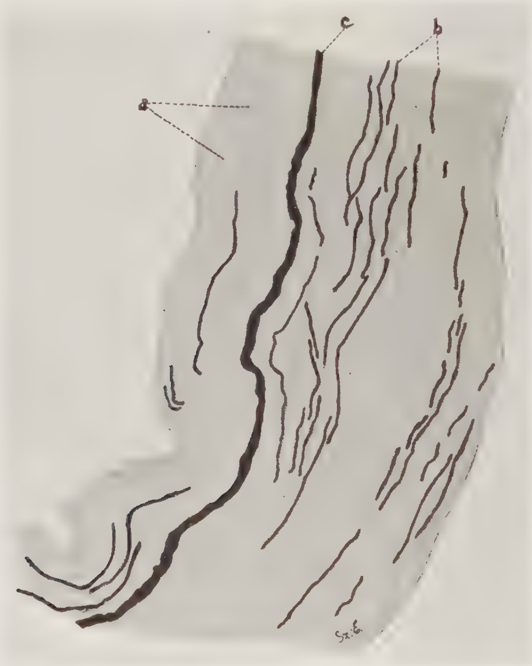

Fig. 5 Drawing showing the microscopic appearance, longitudinal, of the main branch from the diaphragmatic plexus. *a*, sympathetic non-medullated fibers; *b*, fibers with thin medullary sheaths; *c*, fibers with thick medullary sheaths.

### NERVUS VAGUS

Our investigations led us to conclude that the vagus takes no part in the sensory or motor innervation of the diaphragm.

### SUMMARY

1. The phrenic nerve is the only motor nerve to the diaphragm. The phrenic nerve supplies the greater part of the midportion of the diaphragm with sensory fibers. The phrenic nerve also brings many sympathetic fibers to the diaphragm, but these take no part in the sensory or motor innervation of the diaphragm.
2. The lower five to six intercostal nerves supply the margins of the diaphragm with sensory fibers.
3. The sympathetic sends fibers to the diaphragm through two channels: *a*) through the phrenic; *b*) through the plexus diaphragmaticus.
4. The sympathetic fibers take no part in the sensory or motor innervation of the musculature of the diaphragm. They have only a vasomotor and perhaps trophic function.

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# AN ANOMALOUS MUSCLE OF THE FOREARM INSERTING INTO THE SHEATH OF THE MEDIAN NERVE

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ONE HELIOTYPE PLATE (ONE FIGURE)

In the course of dissecting the right forearm of an adult white male subject, a superfluous and unusually anomalous muscle was discovered in the superficial flexor layer. Its origin is from two attachments; the more proximal, by a series of tendinous fasciculi to the capsule of the elbow-joint, just above the head of the radius, and the second, by a band of tendon fibers to the lateral surface of the ulna just forward of the attachment of the supinator muscle. To this band-like tendon, near the point where it narrows down to the main body of the proximal tendon, there are attached a few aberrant fibers of the flexor pollicis longus. About 1 cm. distal to the band there are fascial and a few muscle-fiber attachments from the flexor digitorum sublimis group.

The insertion of the anomalous muscle is two-fold: the more prominent and important is tendinous and on the sheath of that branch of the median nerve supplying the lateral portion of the fourth finger and the medial portion of the third finger; the other, smaller and less important, is fibrous and on the deep palmar fascia.

The proximal tendon is 12.6 cm. long. The belly of the muscle is typically fusiform and is 8.4 cm. long. It receives its nerve supply from a branch of the median nerve, which leaves the median at about the level of origin of the volar interosseous nerve. The blood supply is derived from an artery which arises at the crotch formed by the branching of the ulnar and common interosseous arteries. This slender vessel also gives off branches to the flexor digitorum sublimis group. The distal tendon is 5.7 cm. long and becomes bifid near its insertion.

A careful perusal of the literature fails to reveal the report of a similar muscle anomaly.[1] Nor is the muscle known in normal forms of lower vertebrates. It cannot be claimed to be a reversion to an earlier form in the scale of anatomical evolution. Since the morphology of this unusual variant is so nearly perfect and free from connections with other muscles (except to a slight degree), it would appear that it came into being by a chance differentiation of an additional muscle anlage from the general embryonic muscle mass of the forearm. That the muscle should be inserted into an important nerve of the hand is very unusual. This, also, must be thought of as a freak development.

As to its physiology, it appears certain that the anomalous muscle in the living state was a supplementary flexor of the wrist and that it performed this function by pulling on the median nerve. Since the fleshy belly of the muscle is one of considerable magnitude, it appears reasonable to assume that the individual during life felt a decided twinge every time he flexed his wrist and fingers. It is another illustration of how morphological variation may underlie obscure symptoms.

## PLATE 1

### DESCRIPTION OF FIGURE

1 Dissection of the anomalous muscle of the forearm which arises from the capsule of the elbow-joint and the ulna and inserts into the sheath of a branch of the median nerve. *a*, tendon of origin of anomalous muscle; *b*, fusiform belly of anomalous muscle; *c*, radial artery; *d*, median nerve; *e*, nerve of anomalous muscle; *f*, artery of anomalous muscle; *g*, median nerve; *h*, ulnar artery; *i*, tendon of insertion of anomalous muscle; *x*, point of junction of tendon of insertion of anomalous muscle with the sheath of a branch of the median nerve.

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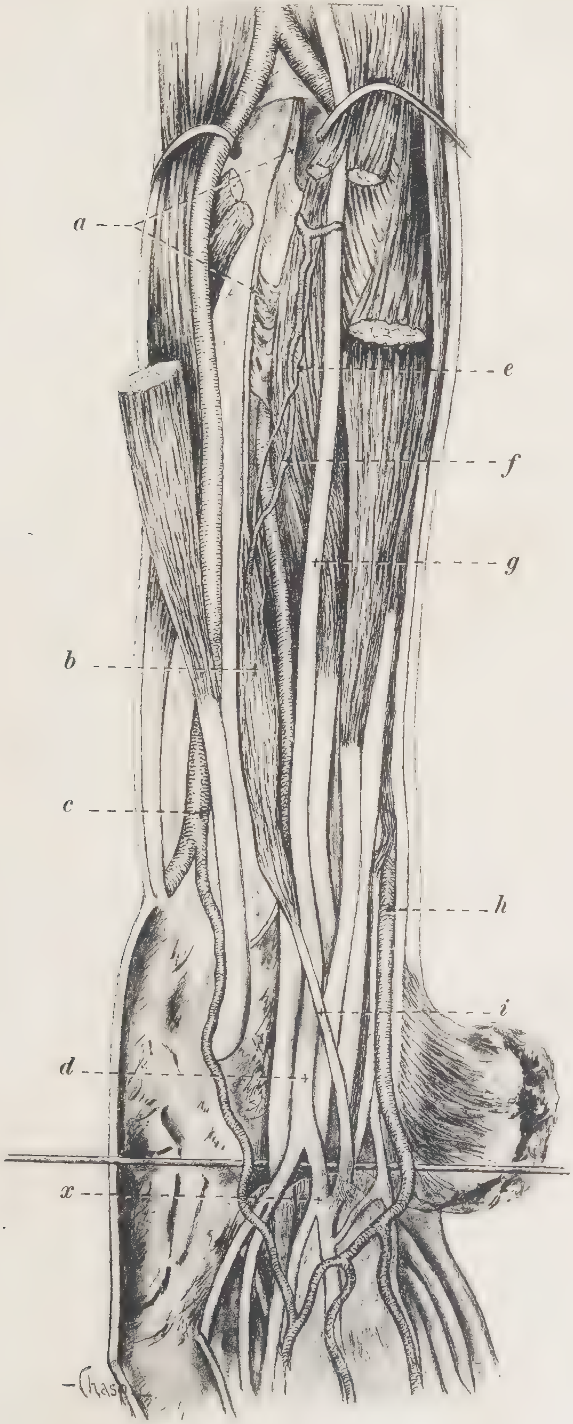
[1] A muscle arising from a nerve, found in a body at the Daniel Baugh Institute of Anatomy, is reported by Dr. Henry K. Seelaus, Anatomical Record, vol. 26, no. 4, p. 307. In his specimen the flexor digitorum sublimis for the fifth finger arose wholly and in a fleshy manner from the sheath of the median nerve and inserted in a manner normal for the sublimis tendons.

ANOMALOUS MUSCLE OF THE FOREARM

JAMES TORRANCE RUGH, JR.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# THE EFFECT OF CASTRATION UPON THE SIZE OF THE PARATHYROID GLANDS AND UPON THE SUSCEPTIBILITY TO TETANIA PARATHYROPRIVA IN THE ALBINO RAT

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## INTRODUCTION

In 1924, M. M. Hoskins and S. B. Chandler began a series of parathyroidectomy operations on albino rats. The results of the operations, as yet unpublished, indicated that before, during, and for a short period following puberty the albino rat is markedly susceptible to tetania parathyropriva. Puberty occurs in the rat at about sixty days of age. Their series included forty-seven operations—twenty-five on rats from fifty to eighty days of age and the remainder on animals eighty to one hundred days old. The proportion of fatal tetania parathyropriva in the former group was 82 per cent and in the latter, 45 per cent. On the basis of these results, it was suggested that a study be made of the effect of castration in the albino rat on the size of the parathyroid glands and on the susceptibility to tetania parathyropriva.

In the rat there are only two parathyroid glands. Zuckerkandl ('03) showed that in this animal only the third pharyngeal pouches give rise to parathyroid anlagen. More recently, Rogers ('27) has shown that the rat never develops a fourth pouch—the pouch designated by Zuckerkandl as the fourth being in reality the ultimobranchial body. The two glands of the rat are very constant in position. They are embedded either partially or completely in the thyroid lobes near the dorsolateral border of the superior pole.

Accessory parathyroid glands have been reported in the human (Kurokawa, '25), in dogs (Marine, '14), and in cats (Shapiro and Jaffe, '23; Nicholas and Swingle, '23). Erdheim ('06), in a series of ten rats, three of which were wild grays and the rest albinos, made serial sections of the neck regions, including the thymus, and reported that accessory parathyroids were found in every case. They were usually embedded in the thymus, and less frequently in the connective tissue between the thyroid and the thymus. Toyofuku ('11) reported that in one rat the number of accessory parathyroids was eleven. On the other hand, Farner and Klinger ('20), in an extensive study of the effect of parathyroidectomy in rats, reported that in only two of sixty-four cases were accessory parathyroids found.

Hoskins and Chandler ('25) have made a thorough investigation regarding accessory parathyroids in the albino rat. Their material consisted of the neck regions of thirty embryos, nine animals ranging in age from birth to thirty days, thirty-nine adult animals, and fifteen cases of the thymus only. Five accessory glands were found out of the total of seventy-nine neck regions, which indicates that one may expect about 6 per cent of these animals to possess accessory parathyroid tissue. They state that they have never found a specimen with more than one accessory parathyroid, and in four of the five cases this one lay cephalad to the thyroid and dorsal to the cartilage of the larynx. In the fifth case the accessory was deeply embedded in the thyroid. It is interesting to note that no accessory parathyroids were found in the thymus. In conclusion, they state:

> It seems to us, after a study of these sections, that the rat furnishes very favorable material for the study of the results of parathyroidectomy. . . . We feel sure that in a long series of experiments with rats one could count on having only a small proportion of the results vitiated by the presence of accessory parathyroids. Moreover, it is reasonable to suppose that the percentage of accessories would be the same in any two large groups so that in experiments comparing the effect of parathyroidectomies under different conditions the question of accessories may be disregarded.

The results of the present study support the belief that accessory parathyroid tissue is not common in the rat. This is, no doubt, due to the fact that there are only two parathyroid anlagen in the rat, while in other species, as pointed out by Marine ('14), the presence of four separate anlagen enhances the possibilities for abnormal distribution outside the thyroid area. Those who have found high numbers of accessory parathyroids in the rat may perhaps have mistaken for them somewhat similar-appearing structures, such as small isolated sections of serous glands or thymic tissue.

Silvestri ('10) reported that previous removal of the ovaries in adult guinea-pigs and dogs protected the animals from the effects of thyro-parathyroidectomy. Massaglia ('11) could not confirm Silvestri's work. He found that previous castration—about twenty days before thyro-parathyroidectomy in three dogs (one, six, and twelve years old, respectively)—did not protect the animals from tetany and death. Also in two female dogs (two and seven years old) removal of the ovaries three to four weeks previous to thyro-parathyroidectomy had no ameliorating effect on the parathyroid-deficiency symptoms. He concluded that removal of the gonads does not prevent tetania parathyropriva and that Silvestri had not performed complete parathyroidectomy in his castrated animals. Clèret and Gley ('11) also reported that previous removal of the ovaries in adult dogs and rabbits had no effect on the results of subsequent thyro-parathyroidectomy.

No record of the effect of castration on the size of the parathyroid glands has been found in the literature. Gates, Grant, and Stevens, in a personal communication, state that castrated rabbits subjected to ultraviolet light do not show enlargement of the parathyroid glands. Grant and Gates ('24) found that if normal rabbits were exposed to ultraviolet light an enlargement of the parathyroids was obtained.

## MATERIAL AND METHODS

Male albino rats were used in this investigation. They were given a diet consisting of bread, cracked corn, milk, lettuce, and meat.

In performing parathyroidectomy in the albino rat, a midventral incision was made in the neck and the thyroid gland exposed. The operation was then continued under glasses which magnified the field about three times. A parathyroid gland could usually be seen as a small grayish body partially embedded in the reddish thyroid tissue along the dorsolateral border of the superior pole of each lateral lobe. Having been located, each gland was then grasped with a pair of fine-pointed forceps and excised by means of iridectomy scissors, together with the thyroid tissue immediately surrounding it. When no trace of the parathyroid could be seen, we adopted the practice of removing the thyroid lobe on that side, in the belief that the parathyroid was completely embedded in the thyroid tissue and therefore not visible by a superficial inspection. After the ensuing hemorrhage had been controlled by cotton packs, the incision was closed with silk thread and washed with 80 per cent alcohol. Infection was not observed in any of the cases.

The excised tissue was then fixed in Bouin's fluid, cut serially at 10 μ, stained with hematoxylin and eosin, and examined microscopically. If two complete parathyroid glands were not found, the operation was called incomplete and the animal discarded.

Two methods of securing the glands for size determination were used. In the first set of experiments dealing with the effect of castration on the size of the parathyroid glands, the latter were obtained by removing from the neck a block of tissue consisting of the trachea and esophagus cut at the superior and inferior levels of the thyroid, together with the thyroid and immediately adjacent connective tissue and muscles. In the second set of experiments, the object of which was to study not only the effect of castration on the size of the parathyroid, but also the susceptibility of such castrated animals to tetania parathyropriva, the glands were obtained by parathyroidectomy.

The method of determining the relative size of the parathyroid glands was as follows: The neck-region tissue and the material removed in the parathyroidectomies were embedded in paraffin, cut serially at 10 μ, and stained with hematoxylin and eosin. The outline of every fifth section of both parathyroid glands of a given individual was then drawn under an Edinger projection apparatus at a magnification of eighty. The total area of these outline drawings was determined in square centimeters by means of a planimeter. The figure thus obtained was regarded as the area of the reconstruction and as representing the size of the parathyroid gland. By dividing the area of the reconstruction by the body weight of the animal, a figure was obtained which was designated the index of the reconstruction and represents the relative size of the parathyroid gland. The probable errors of the average figures of the area and of the index of reconstruction were calculated in each case.

TABLE 1

*Number of litters and animals used in determinations of the size of the parathyroid*

| AGE SERIES | NUMBER OF LITTERS | TOTAL NUMBER OF ANIMALS | NUMBER OF CASTRATES | NUMBER OF CONTROLS |
|---|---|---|---|---|
| 60-day | 27 | 83 | 44 | 39 |
| 75-day | 18 | 58 | 29 | 29 |
| 90-day | 26 | 67 | 34 | 33 |
| Total | 71 | 208 | 107 | 101 |

A comparison of the size of the parathyroid between the castrated and control animals was made at three ages: 1) sixty days, 2) seventy-five days, 3) ninety days. The number of litters and animals in each age series is shown in table 1.

As nearly as possible, half of the members of each litter were castrated and half kept for controls. The body weight was taken at the time of castration and the controls were also weighed at the corresponding time. The animals were again weighed at the time when the size of the parathyroid was determined. The increase in body weight was thus ascertained for both castrated and control animals.

The effect of the removal of the parathyroid glands was compared between castrated and control animals in two age series: 1) sixty days, 2) ninety days.

As shown in table 2, of the total of 143 animals in which the operation of parathyroidectomy was performed, only 113 were taken as being completely parathyroidectomized. Of the thirty animals discarded, twenty-four were incompletely parathyroidectomized, as shown by the histological examination of the tissue removed. Two died from hemorrhage subsequent to the operation. The remaining four exhibited no symptoms whatever during an average time of sixty-three days after the operation, although the tissue removed in each case showed that the usual two parathyroid glands had been completely excised. These animals were killed, and on histological examination of the neck region an accessory parathyroid gland was found in each case. In each of the four cases this accessory gland was located in the position described by Hoskins and Chandler ('25), i.e., cephalad to the thyroid gland and dorsal to the cartilage of the larynx. In one of the cases a small second accessory parathyroid gland was found deeply embedded in the thyroid tissue.

TABLE 2

*Number of litters and number of animals used in parathyroidectomy operations*

| AGE SERIES | NUMBER OF LITTERS | NUMBER OF OPERATIONS | | NUMBER OF ANIMALS COMPLETELY PARATHYROIDECTOMIZED | |
|---|---|---|---|---|---|
| | | Castrates | Controls | Castrates | Controls |
| 60-day | 12 | 34 | 33 | 28 | 26 |
| 90-day | 22 | 35 | 41 | 29 | 30 |
| Total | 34 | 69 | 74 | 57 | 56 |
| | | 143 | | 113 | |

After parathyroidectomy, the animals were closely observed and classified in four groups according to the degree of tetania parathyropriva exhibited by each animal. The classification was as follows: 1) *fatal tetany*—animals dying in marked parathyroid tetany within three days (in one case five days) after the operation; 2) *severe tetany*—animals surviving marked tetany convulsions occurring within three days after parathyroidectomy; 3) *mild tetany*—animals showing only mild intermittent tetany; 4) *no tetany*—animals in which tetany was never observed.

Sixteen of the animals that did not die in fatal tetany were killed at an average time of thirty-one days after the operation. The remaining lived for various lengths of time after the operation, the average length of life being given in data to follow. The completeness of the operation in the animals that did not die in fatal tetany was proved by one of two methods: 1) histological examination of the neck region for accessory parathyroid tissue was shown to be negative; 2) observance of decalcification of the incisor teeth, which is typical of parathyroid deficiency in the albino rat.

## PRESENTATION OF DATA

### *The effect of castration on the size of the parathyroid glands*

Table 3 gives the average figures obtained for the three age series on parathyroid size.

In the sixty-day-age series seven of the animals were castrated at forty days of age and the remaining thirty-seven at twenty days. The figures of this age series show in a very striking and conclusive way that there is no difference in the size of the parathyroid glands between the castrated and control animals. They further show that castration did not affect the normal growth in body weight.

In the seventy-five-day-age series two of the animals were castrated at forty days of age and two at thirty days; the remaining twenty-five were castrated at twenty days. It will be noticed that the average body weight in both the castrates

TABLE 3

*Average figures for the three age series on parathyroid size*

| | CASTRATES | | | | CONTROLS | | | |
|---|---|---|---|---|---|---|---|---|
| Age series | Number of animals | Body weight | Area of reconstruction | Index of reconstruction | Number of animals | Body weight | Area of reconstruction | Index of reconstruction |
| | | *Grams* | *Sq. cm.* | | | *Grams* | *Sq. cm.* | |
| 60-day | 44 | 111.2 | 209.9± 5.0 | 1.941±.05 | 39 | 110.9 | 211.6±6.0 | 1.962±.06 |
| 75-day | 29 | 106.3 | 299.4±14.2 | 3.502±.27 | 29 | 105.5 | 305.2±9.9 | 3.402±.20 |
| 90-day | 34 | 144.4 | 264.6± 3.9 | 1.908±.07 | 33 | 142.1 | 298.4±8.2 | 2.284±.12 |

and controls is much below the normal. This was due to the season of the year, as it was during the summer season extending from late May to early September that the parathyroids of these animals were obtained, and during the summer months a decrease in body weight is usual. The low average body weight causes in part, at least, the abnormally high average indices of reconstruction shown for this age series. There is no difference, however, between castrated and control animals in the average body weight or in the average area and average index of reconstruction of the parathyroid glands.

In the ninety-day-age series all of the animals were castrated at twenty days of age. The average body weight in the castrates and controls is practically the same, being 144.4 grams in the former and 142.1 grams in the latter. In this series, however, the average size of the parathyroid as measured both by the area and the index of reconstruction is greater in the controls than in the castrates. Table 4 shows these differences with their probable errors.

TABLE 4

*Differences between castrates and controls in average area and index of reconstruction for the ninety-day series*

| GROUP | AREA OF RECONSTRUCTION | INDEX OF RECONSTRUCTION |
|---|---|---|
| | *Sq. cm.* | |
| Controls | 298.4 ± 8.2 | 2.284 ± 0.12 |
| Castrates | 264.6 ± 3.9 | 1.908 ± 0.07 |
| Difference | 33.8 ± 9.0 | 0.376 ± 0.14 |

### *The effect of season on the size of the parathyroid glands*

Although the purpose of this study was not to investigate the effect of season on the size of the parathyroid, nevertheless it is considered advisable to mention what little data have been accumulated on this point. No detailed evidence as to a seasonal variation in the size of the parathyroid can be adduced from the data obtained in this investigation. Such evidence would require measurement of the size of the parathyroid in a large number of animals during each month of the year. Only summer and winter differences can be considered here.

In the sixty-day-age series there is not a sufficient number of summer animals in either the castrated or control group to permit a mathematical comparison between winter and summer figures.

The seventy-five-day series is a summer one. The high index of parathyroid size occurring in this series (table 3) is, no doubt, largely due to the abnormally low average body weight. At any rate, this factor precludes the use of this index in any mathematical comparison. The average actual size of the parathyroid in this series as represented by the area of reconstruction cannot, of course, be directly compared with the other age series, but it may indicate an increase in parathyroid size due to season.

TABLE 5

*Comparison of average figures in the summer and winter size determinations of the ninety-day series*

| | NORMAL ANIMALS | | | | CASTRATED ANIMALS | | | |
|---|---|---|---|---|---|---|---|---|
| Season | Number of cases | Body weight | Area of reconstruction | Index of reconstruction | Number of cases | Body weight | Area of reconstruction | Index of reconstruction |
| | | *Grams* | *Sq. cm.* | | | *Grams* | *Sq. cm.* | |
| Summer | 19 | 142.2 | 329.4±15.0 | 2.446±.17 | 16 | 142.0 | 295.6±11.5 | 2.127±.09 |
| Winter | 14 | 142.0 | 256.4± 7.0 | 2.064±.19 | 18 | 146.6 | 237.1± 5.0 | 1.713±.09 |
| | Difference | | 73.0±16.5 | 0.382±.28 | Difference | | 58.5±12.7 | 0.414±.13 |

The ninety-day series is about equally divided between summer and winter material, and a comparison can be made between summer and winter figures, although the number of cases becomes rather small for conclusive mathematical evaluation. The summer group is made up of material obtained during the months of May, June, and July; and in the winter group is placed material secured during the months of October, November, January, and February. Table 5 shows the figures obtained in this comparison. It will be seen that in both normal and castrated animals the average area of reconstruction is greater in summer than in winter. In the normal animals the summer average area of reconstruction is 73.0 sq.cm. greater than the winter average (28 per cent increase), with a probable error of ± 16.5. This difference is 4.4 times its probable error. In the castrated animals the summer average area of reconstruction is 58.5 sq.cm. greater than the winter average (24 per cent increase), with a probable error of ± 12.7. This difference is 4.6 times its probable error. As to the average index of reconstruction, that of the summer castrated animals is 0.414 greater than that of the winter group, with a probable error of ± 0.13. This difference is three times its probable error. In the normal animals, however, the summer index is 0.382 greater than the winter index, with a probable error of ± 0.28. This difference, therefore, cannot be considered mathematically significant.

### *The effect of castration on the susceptibility to tetania parathyropriva*

Table 6 gives the results obtained in the two age series. In the sixty-day series the figures show conclusively that castration did not affect the susceptibility to tetania parathyropriva. In the ninety-day series the differences in the percentages are still not significant. A shift of two animals into the fatal-tetany group, in case of the castrates, or a shift of two animals out of the fatal-tetany group, in case of the controls, would remove any seeming difference in the percentages. In comparing the two age series, no difference in the reaction to parathyroidectomy appears between the sixty- and ninety-day animals. The average length of life after parathyroidectomy in the non-fatal tetany cases is shown to be somewhat longer in the castrates than in the controls both in the sixty- and ninety-day series.

TABLE 6

*Classification of cases of tetania parathyropriva in animals sixty and ninety days of age*

| AGE SERIES | GROUP | NUMBER OF CASES | NUMBER AND PER CENT FATAL TETANY | NUMBER AND PER CENT SEVERE TETANY | NUMBER AND PER CENT MILD TETANY | NUMBER AND PER CENT NO TETANY | AVERAGE SURVIVAL IN DAYS AFTER PARATHYROIDECTOMY IN NON-FATAL TETANY CASES |
|---|---|---|---|---|---|---|---|
| 60-day | Castrates | 28 | 11. 39.2% | 1. 3.5% | 14. 50.0% | 2. 7.1% | 105 (13 animals) |
| | Controls | 26 | 9. 34.6% | 1. 3.8% | 13. 50.0% | 3. 11.5% | 69 (12 animals) |
| 90-day | Castrates | 29 | 8. 27.5% | 4. 13.7% | 15. 51.7% | 2. 6.8% | 90 (18 animals) |
| | Controls | 30 | 11. 36.6% | 4. 13.3% | 11. 36.6% | 4. 13.3% | 48 (13 animals) |

### *The effect of season on the susceptibility to tetania parathyropriva*

Although it was not primarily intended to make a study of the effect of season on the susceptibility to tetania parathyropriva, the data gathered on this point are presented. Only summer and winter comparisons can be made here. The summer series includes those animals that were parathyroidectomized during the months of May, June, July, and August, and the winter series includes those parathyroidectomized during the months of October, November, December, January, and February.

Table 7 shows the figures obtained in comparing summer and winter parathyroidectomies in both the sixty- and ninety-day series. The summer group in the sixty-day series is a small one, but it shows that there is a marked rise in fatal tetany during the summer months. No difference appears between the castrates and controls in either the winter or summer groups. In the ninety-day series, also, there is an increase in fatal tetany in the summer group. No significant difference appears between the castrates and controls in either the winter or summer groups.

TABLE 7

*Comparison of results in summer and winter parathyroidectomies in the sixty- and ninety-day series*

| AGE SERIES | SEASON | GROUP | NUMBER OF CASES | NUMBER AND PER CENT FATAL TETANY | NUMBER AND PER CENT SEVERE TETANY | NUMBER AND PER CENT MILD TETANY | NUMBER AND PER CENT NO TETANY |
|---|---|---|---|---|---|---|---|
| 60-day | Winter | Castrates | 21 | 4. 19.0% | 1. 4.7% | 14. 66.6% | 2. 9.5% |
| | | Controls | 21 | 4. 19.0% | 1. 4.7% | 13. 61.9% | 3. 14.2% |
| | Summer | Castrates | 7 | 7. 100% | .... | .... | .... |
| | | Controls | 5 | 5. 100% | .... | .... | .... |
| 90-day | Winter | Castrates | 14 | 2. 14.2% | 3. 21.4% | 8. 57.1% | 1. 7.1% |
| | | Controls | 12 | 3. 25.0% | 1. 8.3% | 4. 33.3% | 4. 33.3% |
| | Summer | Castrates | 15 | 6. 40.0% | 1. 6.6% | 7. 46.6% | 1. 6.6% |
| | | Controls | 18 | 8. 44.4% | 3. 16.6% | 7. 38.8% | .... |

TABLE 8

*Average index of parathyroid size of all the fatal and severe tetany cases compared with the average index of all the mild and no-tetany cases*

| FATAL AND SEVERE TETANY CASES | | | | MILD AND NO-TETANY CASES | | | | |
|---|---|---|---|---|---|---|---|---|
| Age series | Number of cases | Average body weight | Average index reconstruction | Age series | Number of cases | Average body weight | Average index reconstruction | DIFFERENCE IN AVERAGE INDICES |
| Part A — Castrates | | | | | | | | |
| | | *Grams* | | | | *Grams* | | |
| 60-day | 4 | 100.9 | 1.875±.08 | 60-day | 16 | 104.5 | 1.791±.04 | 1.875±.08 |
| 90-day | 11 | 138.9 | | 90-day | 17 | 153.6 | | 1.791±.04 |
| | (15) | (128.8) | | | (33) | (129.8) | | 0.084±.09 |
| Part B — Controls | | | | | | | | |
| 60-day | 5 | 102.3 | 2.366±.15 | 60-day | 16 | 110.8 | 1.675±.05 | 2.366±.15 |
| 90-day | 15 | 138.2 | | 90-day | 13 | 154.7 | | 1.675±.05 |
| | (20) | (129.2) | | | (29) | (130.4) | | 0.691±.16 |

In the winter groups no significant difference in susceptibility to fatal tetany appears between the sixty- and ninety-day series. In the summer groups the ninety-day animals may be less susceptible to fatal tetany than those sixty days of age. However, the summer sixty-day group is small, and in a larger series the percentage of fatal tetany would no doubt be somewhat lower.

### *Relation of parathyroid size to degree of tetania parathyropriva*

No correlation between the size of the gland and the number of days of survival after parathyroidectomy was found. In table 8 the average index of the size of the parathyroid of all the combined fatal and severe tetany cases is compared with the average index of all the combined mild and no-tetany cases. Part A gives the figures for the castrates and part B, for the controls. In the castrated animals there is no difference in the average index of the size of the parathyroid in fatal and severe tetany cases on the one hand and mild and no-tetany cases on the other. In the control animals, however, there is a significant difference. The average index of the size of the parathyroid in fatal and severe tetany cases is 0.691 (probable error, ± 0.16) greater than the average index in mild and no-tetany cases. This difference is 4.3 times as great as its probable error.

It will also be noticed in table 8 that when the average body weights in the same age series are compared, the mild and no-tetany cases are heavier than the fatal and severe tetany cases in both castrates and controls.

## DISCUSSION OF DATA

The sixty-, seventy-five-, and ninety-day-age series in which the size of the parathyroid gland is compared between castrated and normal animals are sufficiently large for mathematical treatment (table 3). The sixty- and seventy-five-day series show clearly that castration did not affect the size of the parathyroid at these ages. The almost identical averages between castrates and controls in both of these series make this point conclusive. The average indices of the size of the parathyroid in the seventy-five-day series are abnormally high and do not truly represent the relative size of the parathyroid for this age. This is largely due to the fact that a majority of the animals (both castrates and controls) were below normal in body weight, because of a seasonal effect. Since the series is a summer one, the high indices are also probably due, in part, to an increase in the actual size of the parathyroid. The latter cannot be proved, however. The data obtained from the ninety-day series appear, at first, to give a different result (table 4). The average area of the reconstruction of the parathyroid in the controls is 33.8 sq.cm. greater than that of the castrates. This difference is 3.7 times as great as its probable error. However, in the relative size of the parathyroid, as expressed by the index of reconstruction, the average of the controls is 0.376 greater than that of the castrates, which difference is only 2.6 times its probable error. The question now arises as to whether these differences are mathematically significant. The curves of the figures were plotted for both the area and index of reconstruction. In each case the curves were skewed to the right, but the distribution of the figures was much better in the curves of the indices. The index of the parathyroid size is therefore the preferable figure in making the comparisons between castrates and controls. A difference between two averages which is only 2.6 times its probable error cannot, with certainty, be considered to be significant, except perhaps in a perfectly distributed series where normal curves are obtained. This difference, in the two averages under consideration, which is 2.6 times as large as its probable error, would arise by accident alone 7.95 times out of a hundred similar trials.[1] It seems much safer, therefore, to regard this difference as due to chance sampling. In this particular investigation differences due to chance sampling may be very apt to occur, due to a possible seasonal influence on the size of the parathyroid.

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[1] Pearl, Raymond, 1923. Introduction to medical biometry and statistics, p. 218. W. B. Saunders Company.

Regarding the effect of season on the size of the parathyroid, the figures presented are inconclusive, due to the small number of cases. Only the ninety-day animals could be divided into summer and winter groups (table 5). The figures obtained have already been pointed out (p. 312). A seasonal variation in the size of the parathyroid no doubt occurs. It has been shown to occur in the endocrine organs (parathyroids not mentioned specifically) of the rabbit by Brown, Pearce, and Van Allen ('24), and particularly in the case of the thyroid and testis in pigeons by Riddle ('25). The marked rise in the blood-calcium curve during the summer months in pigeons, as shown by Riddle and Reinhart ('26), indicates an increased activity of the parathyroid and possibly an increase in its size during these months. Grant and Gates ('24) found a decrease in the normal size of the parathyroids of rabbits from November to February.

It is considered that the data in this study indicate that an increase in the size of the parathyroid occurs during the summer months as compared with the size found during the winter months. Conclusions as to a differential response between castrates and controls to the seasonal variation in the size of the parathyroid cannot be drawn from the present data. By referring to table 5 it will now be seen that in the ninety-day normal animals there are five more in the summer group than in the winter group, while in the castrates there are two more in the winter group than in the summer. This unequal seasonal distribution of the samples between castrates and controls in the ninety-day-size series may therefore account for the differences in the average figures obtained in this series, as discussed above.

Castration also did not affect the susceptibility to tetania parathyropriva in the two age series studied (table 6). In the sixty-day series the figures in the castrates and controls are practically identical. In the ninety-day series the differences in the percentages obtained are not significant when the size of the series is considered, and when it can be shown that a shift of two animals in or out of the fatal-tetany group will remove the differences. The non-fatal tetany cases in the castrates survived longer, on the average, than similar cases in the controls. Whether this fact is significant or not cannot be said.

The analysis of the effect of season on the degree of susceptibility to tetania parathyropriva shows that fatal tetany increases during the summer months (table 7). This confirms similar results obtained by Hammett ('27). The cause of this summer increase in fatal tetany may be correlated with several factors, such as: 1) a subaverage nutritional status; 2) the actual temperature increase; 3) increase in the size and activity of the parathyroid. Regarding the subaverage nutritional status, Hammett ('27) found the average body weight of rats dying in tetania parathyropriva was less than that of those that survived parathyroid tetany. This point is confirmed in the present investigation (table 8). During the hot summer season a decrease in body weight was observed in many of the animals used in this work. The actual increase in temperature may aggravate the symptoms. Dragstedt, Phillips, and Sudan ('23) showed that tetany could be induced in parathyroidectomized dogs, that had been kept tetany-free on a carbohydrate diet, by exposure to high temperature. It is probable that a summer increase in the size and activity of the parathyroid accounts largely for the increased susceptibility to fatal tetania parathyropriva.

The analysis of the figures regarding the relation between the size of parathyroid and the degree of susceptibility to parathyroid tetany bears on the above point. It was found that in normal animals the fatal and severe tetany cases possessed, on the average, larger parathyroids than the mild and no-tetany cases (table 8). This difference is 4.3 times as great as its probable error. In the castrated animals this relation did not hold, the average index of the parathyroid being the same in both classes of reaction. This latter fact may be due to the insufficiency of the data or to the fact that castration has disturbed the normal size relationship between the size of the parathyroid and the degree of susceptibility to tetania parathyropriva. Of interest in this connection are the unpublished results of Gates, Grant, and Stevens. They found that if castrated adult rabbits were subjected to ultraviolet light there was no enlargement of the parathyroids. Exposure of normal rabbits to ultraviolet light causes an increase in the size of the parathyroids (Grant and Gates, '24). It seems safe to conclude, however, that in normal animals high susceptibility to fatal and severe tetania parathyropriva is correlated with large-sized parathyroids.

Finally, the results obtained in this investigation and the unpublished results obtained by Hoskins and Chandler will be compared. This refers to normal animals. Their series of parathyroidectomies consisted of forty-seven operations, twenty-five on rats from fifty to eighty days of age and the remainder on animals eighty to one hundred days old. Fatal tetany occurred in 82 per cent of the animals in the former group and in 45 per cent of the latter group. All of these operations were performed during the warm spring months of the Arkansas climate. In this study the proportion of fatal tetany was about 35 per cent in both the sixty- and ninety-day series (table 6). These series, however, were made up of unequal proportions of winter and summer operations. The sixty-day series was preponderantly composed of winter operations which would lower the per cent of fatal tetany. The ninety-day series was about equally divided between summer and winter operations. If the summer sixty-day group is compared with the summer ninety-day group (table 7), it is seen that the percentages of fatal tetany correspond in general with those obtained by Hoskins and Chandler in their two age groups. Thus the influence of age on the per cent of fatal parathyroid tetany may be strongly modified by a seasonal effect. This influence of season on the susceptibility to tetania parathyropriva cannot be too strongly emphasized.

## SUMMARY AND CONCLUSIONS

1. The size of the parathyroid gland was compared between castrated and control animals in three age series: 1) sixty days, 2) seventy-five days, 3) ninety days. Castration does not affect the relative size of the parathyroid to a mathematically significant extent in any of the series.

2. The size of the parathyroid gland was compared between winter and summer groups in the ninety-day series. The data indicate an increase in the size of the parathyroid during the summer season as compared with winter.

3. The susceptibility to tetania parathyropriva was compared between castrated and control animals in two age series: 1) sixty days, 2) ninety days. No significant difference in susceptibility appears between the castrates and controls in either of the series.

4. The data on the susceptibility to tetania parathyropriva were analyzed for a seasonal effect. It was found that susceptibility to fatal tetany increases during the summer season, as compared with winter, in both castrates and controls.

5. The data were analyzed for a relation between the degree of tetania parathyropriva and the size of the parathyroid. In the normal animals fatal and severe tetany is correlated with a large relative size of the parathyroid. In castrated animals no relation is shown by the data.

6. Animals that show fatal and severe tetany are, on the average, of lower body weight than those that show mild and no tetany.

I desire to thank Prof. M. M. Hoskins for many helpful suggestions and criticisms throughout this work. I also take pleasure in acknowledging the help of Prof. Eric Ponder in the statistical analysis of the data.

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# RATE AND CHARACTER OF THE COMPENSATORY RENAL HYPERTROPHY AFTER UNILATERAL NEPHRECTOMY IN YOUNG ALBINO RATS

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TWO FIGURES

Various investigators (Morel and Verliac, Carnot, Hinman) have found after unilateral nephrectomy a rapid enlargement of the remaining kidney during the first week or two following the operation in various animals. This preliminary enlargement, or pseudohypertrophy, has been ascribed to a sudden congestion or increase in renal fluids, which soon subsides and is followed by the more gradual increase in renal weight, representing the true compensatory renal hypertrophy. Fiori ('01) and Smith and Moise ('27), however, observed in adult animals a progressive increase in compensatory renal hypertrophy, with no rapid initial enlargement. Jackson and Shiels ('27) likewise found no indication of this preliminary pseudohypertrophy after unilateral nephrectomy in young albino rats, but they did not study the changes during the first week after the operation. The present experiment was therefore undertaken to determine whether there is an initial period of rapid or transient increase in renal weight following unilateral nephrectomy in young rats and also whether there are any significant changes in the water content of the kidneys during the experiment.

## MATERIAL AND METHODS

The albino rats (Mus norvegicus albinus) used in the experiment were reared in the colony at the Institute of

Anatomy, from stock originally obtained at The Wistar Institute of Anatomy, Philadelphia. The diet was a mixture of casein 15 per cent, whole-milk powder 10, sodium chloride 0.8, calcium carbonate 1.5, butter 5.2, whole wheat (ground) 67.5. Water was given ad libitum.

Seven litters were used including forty-two rats (in addition to three rats which were later excluded because of hydronephrosis), twenty males and twenty-two females. The sexes, and the litters so far as possible, were divided equally between the test and control groups in pairs which were similar also in body weight.

The operations were performed when the rats were twenty-eight days old (in two cases at thirty days), which was about a week after weaning. In the test rats the operation was a right-sided nephrectomy by lumbar incision under ether anesthesia. Following exposure, the fibrous capsule of the kidney was incised and retracted over the pedicle, which was ligated. The pedicle was then cut distal to the ligature and the kidney was removed. The lumbar muscles and skin were sutured separately, and collodion was applied to protect the wound. The control rats were similarly subjected to the ether anesthesia, lumbar incision, and exposure of the kidney, i.e., to the same experimental procedure as in the test rats, excepting the removal of the right kidney in the latter case.

After the operations, both test and control rats recovered promptly and were returned to their cages. They continued upon the same diet as before, and the wounds healed in a few days. The rats were weighed individually every day.

In order to study the progress of the renal hypertrophy, the rats, both the test animals and the corresponding controls, were killed by ether. One pair was killed each day up to fourteen days, and thereafter every second day up to twenty-eight days after the operation.

At autopsy the wound area and the viscera were carefully examined. In general, they appeared normal, excepting occasional adhesions of the liver or intestine in the region of the original incision. As above mentioned, three cases were excluded from the series on account of hydronephrosis.

After gross examination and weighing, the kidneys were in some cases (one of the kidneys in each control rat and in every second test rat) cut transversely in halves with a razor, one part being fixed in 10 per cent formalin for microscopic study, the other part being used for drying. In the other cases the entire kidney was used for drying. There was no apparent difference in the water content, whether the half or the whole kidney was used. To determine the dry weights (and water content), the kidneys were dried for three weeks or more to constant weight in open weighing bottles, kept in an electrically heated oven at 95° to 100°C. In a few cases the dried kidneys were lost through accident—which explains the blanks in table 1. Frozen sections were made of the formalin-hardened material, and were stained with hematoxylin-eosin (in some cases with scarlet red) to determine whether the kidneys were entirely normal in structure.

## BODY WEIGHT

Figure 1 shows the average body weight of the test and control groups of rats during the experiment, in comparison with the Wistar norm (Donaldson-King) for corresponding age. The number of rats in each of these groups decreases progressively from twenty-one on the first day to one on the twenty-eighth day, when the last pair was killed. The average body weight in the test group increased but slightly (0.7 gram) on the first day after the operation, but thereafter the growth was remarkably regular. The control group (subjected to operation, but without removing the kidney) increased steadily from the beginning, and their growth curve throughout remained remarkably close to that of the test group. In general, the average body weight increased from about 58 grams at the beginning to 200 grams in the test rats and 188 grams in the controls at the end of the experiment.

It is evident that in these young rats the operation, either with or without removing one kidney, did not appreciably interrupt the growth in body weight. In adult rats, on the contrary, Smith and Moise ('27) noted following unilateral

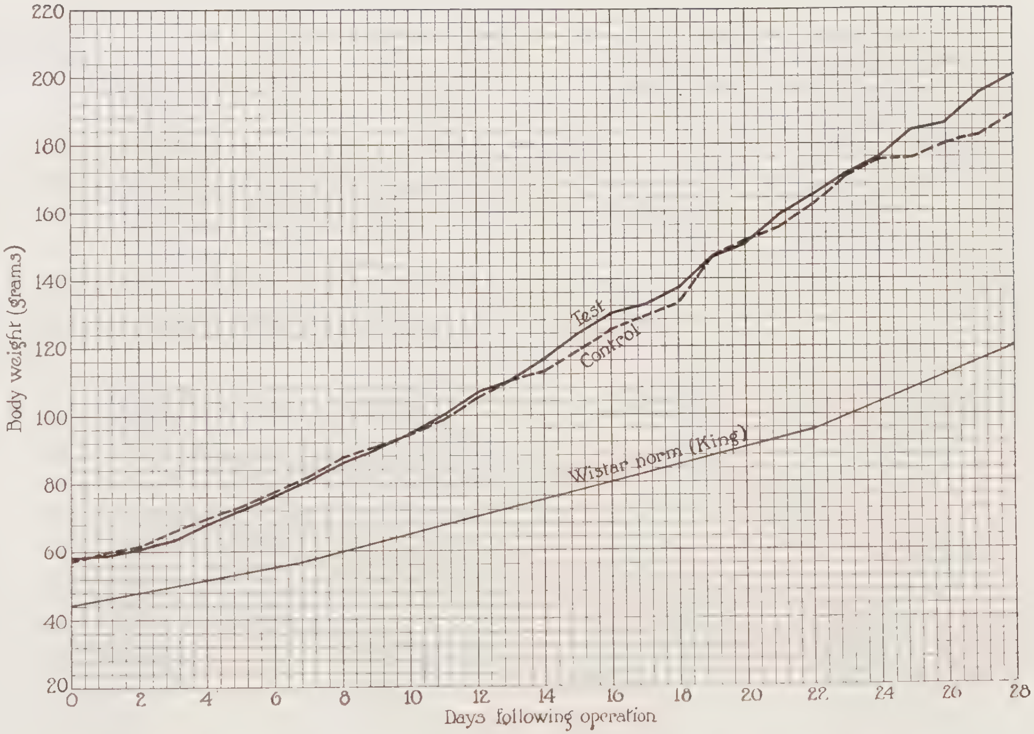

Fig. 1 Graph showing the average body weight of the test rats during the period after unilateral nephrectomy and of the corresponding controls, subjected to operation without removing the kidney. The Wistar norm (from King's data) for body weight (male) according to age during this period is also indicated.

nephrectomy a loss in body weight, which was not regained until after two to four weeks.

In comparison with the Wistar norm (Donaldson's chart 21, for King's data), it is furthermore clear that in the present series the body weight was about one-third above the normal for age at the beginning of the experiment. The subsequent growth was also above the normal rate, so that the rats (both tests and controls) were about 50 per cent above normal weight toward the end of the experiment, as shown in figure 1.

## KIDNEY WEIGHT

### *Fresh weights*

In control rats, as shown in figure 2, the weights of the right and left kidney in each rat were closely alike, although the right was usually slightly heavier. The average excess of the right kidney was 3.94 per cent, which is in general agreement with the findings of Jackson ('25) and Arataki ('26). It is remarkable that during the first six days there was apparently no significant change in the kidney weights of the control rats. This retardation was probably caused by the operation, especially the anesthetic, although it did not prevent an increase in body weight, as above mentioned, during the corresponding period.

In the test rats (fig. 2) the average weight of the twenty-one right kidneys, removed at operation, was 0.384 gram. We may assume that the corresponding average initial weight of the left kidney was about 4 per cent less, or 0.369 gram. In the test rat killed one day after the removal of the right kidney, the remaining kidney showed practically the same weight as the left kidney of the control. Thereafter, the left kidney showed a variably progressive hypertrophy, in comparison with the left kidney in the control rat of the same age. There is evidently some irregularity due to individual variations (especially on the ninth and eighteenth days), but after the first week there is a remarkable correspondence in the increases and decreases of kidney weight in corresponding test and control rats.

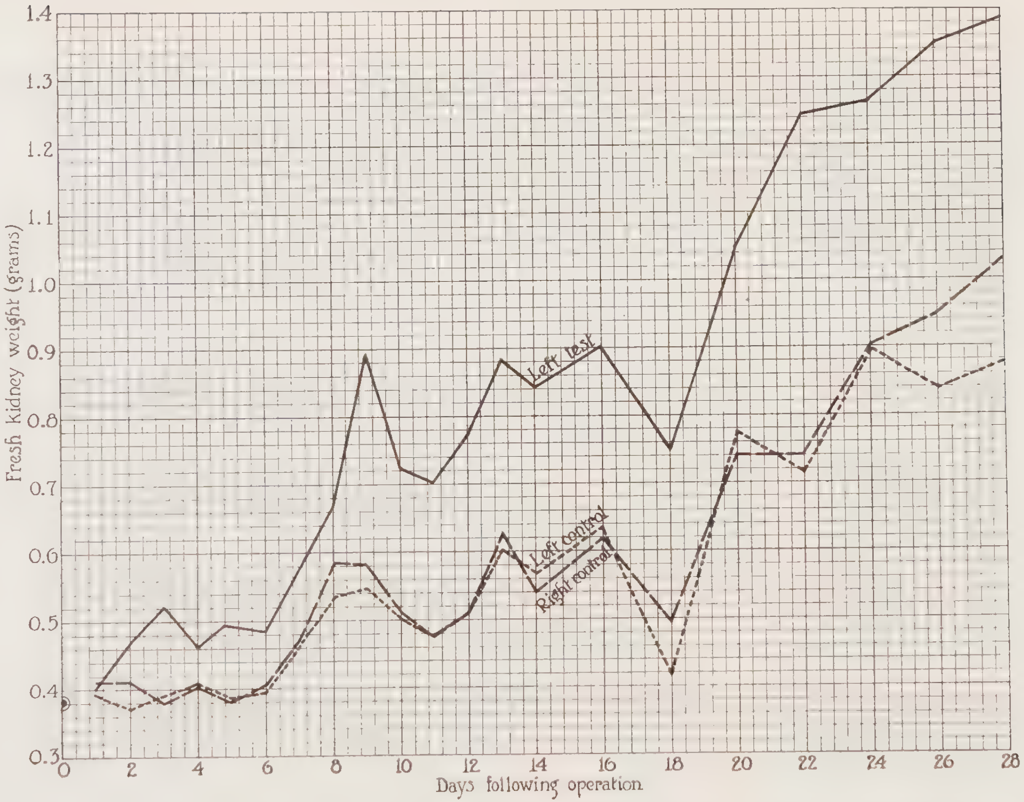

Fig. 2 Graph showing the kidney weights of the rats at various periods. The encircled dot indicates the average weight (0.384 gram) of the twenty-one right kidneys removed at operation from the test rats, twenty-eight days of age. The upper line indicates the weight of the remaining (hypertrophied) left kidney at successive intervals following the operation. The lower two lines represent the weights of the right and left kidneys in the corresponding control rats of the same age, sex, and body weight.

As shown by the growth curve in figure 2, the hypertrophied kidney apparently increased rapidly in weight during the first three days, but decreased somewhat during the remainder of the first week. During the second week the kidney again showed a marked increase in weight which continued somewhat irregularly through the third and fourth weeks of the experiment. The percentage hypertrophy of the kidney was slightly more irregular, as shown in the second column of table 1. However, there was apparently a rapid increase to

TABLE 1

*Percentage hypertrophy in weight of the left kidney (fresh weight, compared with left control) and percentage of dry substance in each kidney at various days following the operation*

| DAYS FOLLOWING OPERATION, AND SEX OF RATS | PERCENTAGE HYPERTROPHY OF LEFT KIDNEY AFTER REMOVAL OF RIGHT | PERCENTAGE OF DRY SUBSTANCE IN KIDNEY | | | |
|---|---|---|---|---|---|
| | | In test rats | | In control rats | |
| | | Right removed at operation | Left removed at autopsy | Right removed at autopsy | Left removed at autopsy |
| 1 f | 0.40 | 20.56 | 21.18 | 20.33 | 21.27 |
| 2 f | 26.61 | 18.52 | 21.91 | 22.28 | 22.78 |
| 3 m | 34.89 | 22.12 | 19.51 | 18.97 | 21.76 |
| 4 f | 12.90 | 19.85 | 22.68 | .... | 20.86 |
| 5 f | 28.63 | .... | 21.41 | 21.68 | .... |
| 6 f | 23.19 | 21.85 | .... | 20.93 | 21.46 |
| 7 f | .... | 20.65 | .... | 21.38 | 19.84 |
| 8 m | 26.02 | 19.79 | 20.98 | 19.37 | 19.90 |
| 9 f | 63.98 | 19.56 | 20.12 | 21.18 | 22.15 |
| 10 f | 44.78 | .... | 22.09 | 20.54 | 20.90 |
| 11 f | 47.67 | 22.46 | 20.51 | 21.51 | 22.02 |
| 12 m | 50.64 | 20.58 | 22.89 | 21.59 | 21.24 |
| 13 f | 46.44 | 21.42 | 20.55 | 21.87 | 22.18 |
| 14 f | 49.25 | 21.78 | 19.64 | 21.51 | 21.76 |
| 16 m | 43.94 | 20.92 | 19.54 | 20.40 | 20.32 |
| 18 m | 80.80 | 21.16 | 22.50 | 19.06 | 19.67 |
| 20 m | 35.06 | 20.18 | 21.90 | 21.08 | 21.79 |
| 22 m | 73.97 | 20.95 | 22.49 | 22.58 | 21.73 |
| 24 m | 41.56 | 21.87 | 23.70 | 22.28 | 22.62 |
| 26 m | 61.02 | 21.10 | 22.64 | .... | 22.89 |
| 28 m | 58.60 | 20.44 | 22.72 | 23.08 | 23.33 |
| Mean | | 20.83 | 21.52 | 21.14 | 21.52 |
| Standard deviation | | 0.963 | 1.24 | 1.12 | 1.01 |
| Probable error | | 0.15 | 0.19 | 0.17 | 0.15 |

about 35 per cent hypertrophy at the end of the third day, then decreasing to a lower level, being about 13 per cent on the fourth day. Thereafter, the percentage hypertrophy increased irregularly throughout the period of the experiment. The final hypertrophy at the end of the fourth week was about 60 per cent. If Donaldson's norm (for kidneys at corresponding body weight) were used for comparison instead of the control left kidneys, the apparent renal hypertrophy would be much greater, because the control kidneys are themselves somewhat above Donaldson's norm.

The results of the present experiment are thus in general agreement with the theory of an early period of pseudohypertrophy (congestion), followed by subsidence and subsequent progressive true renal hypertrophy. However, a larger number of cases, especially during the first week, would be necessary to establish this conclusion with certainty. The present result might possibly be due merely to chance variation among the relatively few individuals.

### *Dry weights and water content*

As shown in table 1, the dry substance in the normal right kidney (removed at operation) of the test rats averaged 20.83 ± .15 per cent. In the remaining hypertrophied left kidney, removed later at autopsy, the dry substance averaged 21.52 ± .19 per cent, or 0.69 per cent greater. The probable error of the difference of the two means (obtained by taking the square root of the sum of their probable errors squared) is 0.24 per cent; so that the difference, being slightly less than three times the probable error of the difference, is of doubtful significance. A slight relative increase in dry substance of the kidney with age might be expected normally (see Lowrey's data, cited by Donaldson).

To determine whether there has been any change in the water content of the kidney as a result of the compensatory hypertrophy, the best comparison is between the hypertrophied left kidney of the test rats and the normal kidney of the control rats of the same age and body weight. As is apparent from table 1, the mean relative dry weight in the left control kidney was 21.52 per cent, or exactly the same as in the hypertrophied test kidney, while that in the right control (21.14 per cent) is not significantly different. Therefore, the conclusion is that there has been no significant change in the relative dry substance (or water content) of the hypertrophied left kidneys of the test rats.

Since there is no marked change in the water content of the hypertrophied kidneys, it is clear that the increased weight could not be due merely to an increased amount of water or watery liquid (such as urine) containing very little solids. This is confirmed by the microscopic study of the sections, which show no indication of edema or dilatation of the uriniferous tubules. Smith and Moise ('27) found a definite increase in the relative water content of the rat's kidney during late stages of compensatory hypertrophy on high-protein diet, but not much change during the earlier stages. They record no observations for the water content on normal diet. Cameron and Kellaway ('27) found no appreciable increase in the water content of the hypertrophied kidney in young guinea-pigs after unilateral nephrectomy or ureteral ligation.

The lack of any definite change in the water content of the hypertrophied kidneys in the present series might be thought to exclude also the possibility of a renal hyperemia, but such is not the case. Donaldson's data for the water content of the rat's blood indicate that it is only about 2 per cent above that of the kidney at body weights corresponding to those of the present experiment. It therefore follows that an increase in blood sufficient to raise the renal weight one-third would increase the relative renal water content only about 0.66 per cent, which would appear insignificant in the present series.

No detailed histological study was made of the kidneys in this series. Hyperemia was frequently observed in the sections, however, in agreement with the observations of Albarran ('99) and Saphir ('27). Jackson and Shiels ('27) observed no excessive renal hyperemia, but their series did not include the first week following the nephrectomy. Since, furthermore, it is very unlikely that any considerable amount of true cellular growth could occur within three days, the conclusion seems justified that the early increased renal weight following unilateral nephrectomy is a pseudohypertrophy to be ascribed chiefly to blood-vascular congestion.

## SUMMARY

1. The right kidney was removed in twenty-one albino rats at twenty-eight days of age. Twenty-one litter-mate controls were subjected to the same operation (anesthetic, incisions, etc.), but without removing the kidney. The subsequent growth in body weight was not appreciably retarded by the operation either with or without nephrectomy.

2. Test rats and corresponding controls were killed and autopsied at successive intervals, daily up to fourteen days and every second day thereafter up to twenty-eight days after operation. The operation with anesthesia apparently prevented the normal growth of the kidney during the first week in the control rats, although the growth in body weight was unaffected. After the first week, the growth of the kidney in the control rats appeared normal. The right control kidney averaged about 4 per cent heavier than the left.

3. In the test rats the remaining left kidney at first increased rapidly in weight, reaching an apparent hypertrophy of about 35 per cent in three days. A decrease in renal weight appeared during the remainder of the first week. Subsequently, there was an irregularly progressive renal hypertrophy, finally reaching about 60 per cent.

4. The dry weights showed no significant differences in relative water content of the hypertrophied left kidneys, in comparison with either the normal right kidneys of the test rats (removed at operation) or the kidneys of the control rats of the same age and body weight.

5. The lack of any appreciable change in relative water content of the hypertrophied kidneys, as well as the histological appearance, excludes the possibility that the increased

renal weight might be due to increased content of urine or other liquid of low solid content. This does not, however, exclude the presence of a marked hyperemia, since the relative water content of the blood is but slightly above that of the kidney.

6. The results are therefore in agreement with the theory that, following unilateral nephrectomy, there is an initial transient pseudohypertrophy of the kidney, due chiefly to blood-vascular congestion, which subsides and is followed by progressive true renal hypertrophy. To establish this conclusion with certainty, however, a larger number of cases is necessary, especially during the first week.

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# THE REACTION OF THE SEGREGATION APPARATUS OF THE THROMBOCYTES AND LEUCOCYTES OF THE BLOOD OF NECTURUS TO NEUTRAL RED AND TO PROLONGED EXPOSURE TO OSMIC ACID

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TWO PLATES (NINETEEN FIGURES)

In a previous paper (Dawson, '28) the possible identification, in the erythrocyte of Necturus, of the segregation apparatus with the Golgi apparatus was suggested. The same methods, the technic of Wright and Giemsa, supravital staining, the technic of Nassonov-Kolatchev, and prolonged exposure to osmic acid in a hanging-drop, have been applied to the thrombocytes and leucocytes of Necturus. All of these several methods, however, were not applied with equal success to each of the six types of cells under investigation. In an earlier study on the blood of the frog, Jordan ('25), using supravital staining with neutral red, demonstrated the presence of a segregation apparatus of granules and globules in every type of blood cell of this animal. The present study of the blood of Necturus also discloses the presence of a similar apparatus in practically every type of cell. In Necturus, however, the elements of the segregation apparatus are not so generally distributed throughout the cytoplasm as in the case of the frog, but usually are grouped and restricted to certain areas of the different cells.

## MATERIAL AND METHODS

Fresh blood, for smears, hanging-drop preparations, and supravital staining, was obtained directly from the branchial circulation by the method described by Charipper ('28).

Blood obtained in this way is free from any contamination by the secretions of the skin and, in addition to this advantage, the same animal may be used repeatedly without injury. In staining smears with Wright's or Giemsa's, the stains were made up according to formula, but the time of staining and dilution were varied to secure the best differential reaction.

In preparing hanging-drop preparations for treatment with osmic acid, plate-glass slides with a 4-mm. well were used. Three to five drops of 2 per cent osmic acid were placed in the well, the cover-slip with the drop of blood inverted over the acid, and the margin of the slip sealed with warm vaselin or paraffin. These preparations were kept at room temperature and inspected regularly until blackening of cell inclusions could be observed, usually a period of from ten to fourteen days. The results obtained with this method were very variable and seemed to depend somewhat on the amount of blood in the drop. The method was also varied by spreading the blood in a thin film and by diluting the blood with a dilute oxalate solution.

The special inclusions of thrombocytes and erythrocytes were readily blackened with osmic acid in practically every case, but great difficulty was encountered in the case of the leucocytes. In films the leucocytes were greatly flattened, and in this condition were never observed to react with osmic acid. When the blood was diluted with oxalate solution, these cells rounded up and, as the exposure to osmic acid proceeded, became so dark as to make it impossible to recognize any intracellular elements. In all cases of success with leucocytes by this method the cells were only moderately flattened and were usually in contact with neighboring cells.

In supravital staining, neutral red alone, Janus green alone, mixtures of neutral red and Janus green, and brilliant cresyl blue were applied according to the method described by Sabin ('23). Neutral red applied supravitally stains the granules of the segregation apparatus only. Janus green used alone stains the mitochondria a bluish green after an interval of fifteen to twenty minutes, but it often stains the segregation apparatus almost immediately or as rapidly as does neutral red. When used in combination, neutral red stains the segregation apparatus rapidly and Janus green the mitochondria more gradually. Brilliant cresyl blue also stains the segregation apparatus.

Preparations stained supravitally were fixed and mounted after the method described by Scott ('28). In some cases it was found that, after fixation by rapid drying, the neutral red also appeared in the mitochondria. This, however, was never observed to occur when neutral red and Janus green were used together. After fixation, it is also to be noted that the Janus green which was confined to the mitochondria often appears in the nuclei, especially of the lymphocytes. When studying supravital preparations, the observer's eyes should be shielded from all light except that coming through the tube of the microscope. This precaution is especially necessary if a clear view of the blue-green chondriosomes is to be secured. Bodies stained with neutral red can be seen with ease under almost any condition of illumination within the room.

In order to compare the pattern of the cell inclusions, demonstrated by supravital staining with neutral red, with structures ordinarily blackened by methods specific for the Golgi apparatus, portions of the mesonephros were prepared by the method of Nassonov-Kolatchev. In using mesonephros it is possible to secure a considerable number of blood cells for study, both in the sinusoids of this organ and in the aorta and vena cava which lie in the middorsal line, in association with the mesonephroi. Very complete blackening of a typical Golgi reticulum was obtained in all portions of this organ, especially in the epithelium of the mesonephric tubules and in the cells of the sympathetic ganglia of this region. But the blood cells and the cells of the interstitial tissue were found to contain blackened inclusions which did not conform to the typical pattern or reticulum. Satisfactory impregnation was usually obtained by treating the tissue with 2 per cent osmic acid at 35°C. for five days. When on the slide, the sections were placed in turpentine for twelve hours and subsequently bleached to the desired intensity with a weak solution of hydrogen peroxide in absolute alcohol.

References to the literature will be omitted at this point and will be postponed largely until the discussion of the results of this study is undertaken. This is done in order to avoid anticipating the observations made and the conclusions reached herein.

## DESCRIPTION OF MATERIAL

The cells of the blood of Necturus include erythrocytes, thrombocytes, and leucocytes. The leucocytes consist of large and small lymphocytes, large mononuclear (spheronuclear) and reninuclear monocytes ('transitional leucocytes'), and eosinophilic, neutrophilic, and basophilic granulocytes (Jordan, '25). The several cell types can usually be readily distinguished in smears stained with Giemsa's or Wright's technic, but it is occasionally difficult to decide whether a cell is a large lymphocyte or a small monocyte. In preparations stained supravitally the individual cells are also usually recognized with ease, but in osmicated preparations the distinguishing tinctorial qualities are gone and identification is sometimes difficult. This is especially true of the lymphocytes and monocytes where the nuclear patterns and cytoplasmic inclusions are much alike. The neutrophiles and eosinophiles can be identified by their specific granulation and nuclear patterns, but the basophilic granulocytes could not be identified with certainty in any of the fixed and sectioned preparations.

### *The thrombocytes*

In stained smears these cells may be elliptical, oval, or rounded in outline. The nucleus is relatively large and stains blue. The cytoplasm is typically accumulated at the opposite poles of the nucleus and is usually reduced to a very small quantity in the remaining portions of the cell. Ordinarily, the cytoplasm of the poles is filled with reddish-purple (azurophile) granules, but some of the granular material often stains blue. The granules are usually concentrated near the ends of the nucleus, with the result that peripherally in these cells there is a hyaline, non-granular, pale-blue margin or ectoplasm. Not infrequently, a vacuole-like area of variable size, situated centrally within the granular regions, may be observed.

When stained supravitally with neutral red and Janus green, the thrombocytes are found to contain numerous, blue-green, granular mitochondria and several small granules which stain intensely with neutral red. Both the mitochondria and the neutral-red granules are restricted to the granular areas of cytoplasm. Occasionally the neutral-red granules are arranged in a circle about a small clear non-granular area, but usually they present no definite pattern of distribution and tend to lie in the vicinity of the poles of the nucleus. Isolated thrombocytes do not appear to stain supravitally as well as those which are in clumps or rosettes. Clumping or agglutination is favored by allowing the drop of blood to stand for a short time before covering and sealing with vaselin.

The thrombocytes found in the blood vessels of the mesonephros which had been prepared by the technic of Nassonov-Kolatchev showed several relatively large, blackened globules. They were usually grouped near the poles, but in cells which have shortened or rounded up, the globules may be irregularly distributed throughout the cell.

In hanging-drop preparations, exposed to the fumes of osmic acid, small densely blackened granules were clearly visible at the end of the fourth day (figs. 10 and 11). They were situated in the granular zone and were of approximately the same size and number as the granules stained with neutral red. Longer exposure to osmic acid usually resulted in the entire granular portion of the cytoplasm becoming so dark that specific elements could not be readily observed even with an oil-immersion lens. Efforts to bleach these cells in order to remove the excess of impregnation were not markedly successful.

### *The lymphocytes*

The lymphocytes of Necturus are essentially similar to those of other Amphibia. They vary greatly in size. The nucleus is usually spherical, but often shows an indentation even in smears, and is always located slightly eccentrically. In films the cytoplasm usually stains a clear blue, but often contains a number of denser irregular blue clumps among which appear a variable number of small round metachromatic granules. The granules tend to be concentrated in a narrow crescent on the side of the nucleus where the amount of cytoplasm is greatest. The area of the centrosphere, however, was never clearly seen in this region.

In all lymphocytes a relatively large number of granular and bacillary chondriosomes, staining bluish green with Janus green, can be seen. They are grouped, as a rule, in the cytoplasmic region where the metachromatic granules are demonstrated in smears. They can, however, be also found in considerable numbers in the cytoplasm on the opposite side of the nucleus.

Even after thirty minutes of exposure to neutral red there are many lymphocytes in which no neutral-red inclusions can be seen. After two hours, the number without stained granules appears considerably less. Of course, it is always possible that the red granules may lie below the nucleus so as to be obscured and that the cells may gradually shift so that the granules are in a position to be observed. The number of granules is exceedingly variable: one, two, and up to eight or ten can be counted in various cells. In other cells it is impossible to count the granules, but the number must be much greater than ten.

In all cells where the granules are numerous they tend to be arranged in a circle or rosette on the side of the nucleus where the cytoplasm is accumulated. These granules appear to remain relatively constant in size even after several hours of staining. The typical distribution just described is characteristic only of the resting or quiescent cell. In cells exhibiting ameboid activity the neutral-red granules are apparently distributed in a haphazard manner.

The lymphocytes from the blood vessels of the tissue impregnated with osmic acid by the method of Nassonov-Kolatchev present a very striking and characteristic picture (figs. 7, 17, and 18). In the cytoplasm in the region of the nuclear indentation a group of densely blackened granules is present. In every case they are grouped about a circular clear area and show varying degrees of fusion, in some cases joined by delicate filaments, in other cases closely approximated to simulate a beaded rod. The arrangement of the granules within a given group is also regular and appears to be a mingling of a concentric with a radial pattern about the circular clear area, presumably the centrosphere. In some cells the radial pattern appears to predominate, in others the concentric arrangement is the more pronounced.

No information could be gained from hanging-drop preparations. The small amount of cytoplasm, the rounding-up of the cell, and the general darkening of the cytoplasm made observation of cytological detail impossible.

### *The monocytes*

The monocytes, in contrast with the lymphocytes, are characterized by an abundant, though less basophilic cytoplasm, accumulated largely on one side of a spherical, oval, kidney-shaped, or deeply constricted nucleus. In cells successfully stained by either Wright's or Giemsa's method a more basophilic zone, usually semicircular in form, is seen adjoining the nucleus (fig. 19). In this region the centrosphere is well developed. About the area of the centrosphere, in many cells, a number of purple-red granules are often demonstrated. Not infrequently these granules have a definite radial arrangement, but in many cells the granules appear to be scattered irregularly through the area of more basophilic cytoplasm.

When stained supravitally with neutral red and Janus green, the monocytes are seen to contain a typical rosette of numerous red-stained granules lying about the centrosphere, while about the rosette is a peripheral fringe of blue-green chondriosomes. In supravital preparations the neutral-red granules exhibit a striking tendency to increase in size and soon assume the appearance of globules, but never acquire the optical properties of vacuoles (fig. 1). During ameboid activity the formed constituents of the cytoplasm may become irregularly displaced throughout the cell.

The picture presented by the monocyte after impregnation with osmic acid (Nassonov-Kolatchev) is very variable. In some cells a group of densely stained separate granules is found in the region of the indentation of the nucleus (fig. 8). In other cells (fig. 2) the blackened granules are arranged as a typical rosette, but exhibit some evidence of clumping and fusion. Again, the granules may be grouped in several clusters joined by slender filaments (fig. 16). In still other cells the material impregnated with the osmic acid is arranged as rods or filaments extending radially from the areas of the centrosphere. The radial arrangement, however, is not always maintained, and the blackened material may appear as curved rods or open loops (fig. 9). The rods or filaments may have a smooth, even contour or may appear beaded. This variability stands in rather striking contrast to the constancy of pattern observed in the lymphocytes.

In hanging-drop preparations occasional cells are found in which some detail can be observed. The osmiophilic material in the few cases observed was arranged as radial filaments.

### *The neutrophiles*

The neutrophiles of Necturus do not present any unusual features in stained smears. Their nuclei show a varying degree of lobulation. Besides the specific granules, other larger and more deeply stained bodies may be present. They are usually grouped: sometimes in a single cluster (fig. 14), at other times in two or more clusters (fig. 12). When found in a single cluster, they often present a radial arrangement. No well-defined clear area, centrosphere, was seen in these cells.

When supravitally stained with neutral red, these cells are found to contain a varying number of small, granular chondriosomes and a definite segregation apparatus, composed of small, red-stained granules which in the quiescent cell usually occupy the center of the cell and are often radially aligned from the centrosphere. As in the monocytes, the granules of the segregation apparatus show a tendency to increase in size on prolonged staining with neutral red and develop the optical properties of globules (fig. 4).

The picture presented after exposure to osmic acid, either by the method of Nassonov-Kolatchev or in hanging-drop, is fairly uniform (figs. 6 and 13). The osmiophilic substance is usually arranged as a rosette of radiating rods or filaments, grouped about a circular, clear area. In some cells the filaments appear entirely separate, but in others they may appear as narrow U-shaped bodies with the arms of the U extending peripherally, resembling in appearance a miniature equatorial plate of chromosomes. Several neutrophiles have been observed, however, in which the osmiophilic substance appeared as a group of blackened globules joined by relatively heavy bridges.

### *The eosinophiles*

The eosinophiles are relatively numerous in the blood of Necturus and in smears are encountered almost as frequently as the neutrophiles. The nucleus is usually irregularly lobed and is generally located at one pole of the spheroidal cell. The rest of the cell is crowded with large spherical granules which usually exhibit a radial arrangement from the region of the centrosphere (fig. 15). Immediately about the clear area of the centrosphere is a group of smaller and more densely stained granules which are also aligned radially.

In supravital preparations the eosinophiles are readily recognized by the extremely eccentric position of the nucleus and the presence of the coarse granules brilliantly stained with neutral red. Near the nucleus there is a relatively clear zone from which the coarse granules are arranged in more or less definite radial lines. No definite segregation apparatus could be distinguished with certainty in these cells. The chondriosomes also were never seen as distinct bodies, but in many cases the tinting of the cell indicated the possible presence of elements of a blue-green color.

In the cells impregnated with osmic acid by the method of Nassonov-Kolatchev the specific granules appear as dark-brown bodies characteristically aligned as radii from a region near the eccentric, lobed nucleus. The osmiophilic substance in these cells appears as either a group of radially arranged rods (fig. 5) or as a cluster of blackened globules connected by broad bands (fig. 3). The material reacting with osmic acid usually surrounds the centrosphere, but in several cells (fig. 5) blackened rod-like bodies may also be scattered through the cytoplasm.

In hanging-drop preparations the specific granules of the eosinophiles react quickly with osmic acid, and in thirty minutes these cells are a dark brown, and in a few hours appear black. Owing to the rapid reaction of the coarse granules with osmic acid, the specific osmiophilic substance has not been recognized in the rounded cell.

### *The basophiles*

These cells are relatively few in number—4 per cent of the leucocytes according to Michels ('23). In smears they appear as round cells slightly smaller than the eosinophiles. The nucleus is usually centrally located and vesicular. The granules are closely packed and give the characteristic color reaction of this type of cell.

Neither chondriosomes nor segregation apparatus were definitely identified. The reaction of the specific granules with neutral red was not uniform, so that it was impossible to be sure whether the red bodies appearing in some cells were specific granules or granules of the segregation apparatus.

In Nassonov-Kolatchev preparations the basophiles were never positively identified. Occasionally finely vacuolated cells with a central round nucleus were encountered. They may have been basophiles from which the granules had been dissolved. In hanging-drop preparations, also, these cells were never identified with certainty.

## DISCUSSION

From the preceding description of the blood cells of Necturus studied by three different methods, it is evident that there are three types of cell inclusions demonstrated which are characteristically grouped in the region of the centrosphere, the so-called azurophile granules, the granules of the segregation apparatus, and the osmiophilic bodies. In thrombocytes, lymphocytes, and monocytes the similarity in size and position of the neutral-red granules and the bodies blackened with osmic acid is striking and immediately suggests that these are identical structures reacting specifically to two different methods of treatment. Maximow ('28) was impressed by this and states:

> In the majority [of lymphocytes] however, a group of neutral red granules or 'vacuoles' is located in the excavation of the nuclear surface. They obviously surround the cytocentrum and their position corresponds to the place where on fixed and impregnated material the Golgi net can be detected. I cannot be affirmative regarding the relations between the neutral red inclusions, the 'vacuome,' and the Golgi net (p. 200).

While Maximow used the term Golgi net, his figure (Maximow, '27, p. 331) shows the 'net' as a cluster of granules which appear, in some instances, to be joined by delicate filaments. Parat, in his discussion of a paper by Lewis ('28, p. 258), offers the following observation:

> Les remarquables préparations du Prof. Lewis montrent a l'évidence, du moins dans les cellules épithelioides et aussi dans les mononucleiaires, la polarization des granules ou éléments du "vacuome" colorables par le Rouge Neutre dans une zone ou se trouve généralement le centrosome. Ou, c'est dans cette région que l'on peut demontrer l'appareil de Golgi, qui ainsi que nous le demontrerons, peut correspondre exactement au vacuome ou aux mitochondries qui l'entourent ou aux lipoids accumulés dans cette région, au contact des éléments du vacuome.

Although the possible identification of the Golgi apparatus with the segregation apparatus has been tentatively suggested, no one apparently has been rash enough to suggest that the azurophile granules should also be regarded as identical with these two structures demonstrated in blood cells. This possibility seems, however, to be worthy of consideration. Nothing is known regarding the significance of the azurophile granules. Maximow ('27, p. 324) suggests that they may be regarded as a visible symptom of a peculiar secretory function or of another metabolic process. This view of Maximow has much in common with the recent hypothesis of Chlopin ('27, '28), who finds that, after exposure to neutral red, many cells are found to contain special bodies which have an affinity for this dye. These bodies, he finds, are not preformed, but are induced by the presence of the vital dye, and, furthermore, appear in fixed preparations, being characterized by a decided basophilia, reacting especially well with thionin-aurantia and eosin-azur. To this substance, the presence of which in cytoplasm can be demonstrated by its ability to react with vital dyes and produce definite bodies, Chlopin gives the name 'Krinomsubstanz.' Frequently, the krinom substance has the same topographical relationships within the cell as the Golgi apparatus, but Chlopin finds many apparent exceptions to this.

In the blood cells, at least, we are not dealing with absolutely induced structures so far as the neutral-red bodies are concerned, since they can be readily seen in many cells in fresh preparations, as Jordan ('25) has already pointed out. The neutral-red bodies, however, increase both in number and size in the presence of this dye, but the globules may increase also in the absence of the dye or, as Jordan ('25) states: "The globule does not enlarge because it stores more stain; on the contrary, it simply 'stores' more stain because it enlarges" (p. 119). In other words, the appearance of these bodies in blood cells is the visible expression of certain reactions occurring in the cytoplasm due to changes in the metabolism of the cell; presumably, in the case of supravital preparations, to changes induced by a changed and unfavorable environment. Accordingly, it is evident that the substance of the segregation apparatus has many properties in common with the krinom substance, including basophilia, which is especially marked in the erythrocytes (Dawson, '28).

While it can be demonstrated that the granules increase in size in the absence of neutral red, one gains the impression that, in some cases at least, the increase in the size and number of the granules is favored by the presence of the dye as well as by the other conditions under which the observations are made (Dawson, '28). It may be that in normal physiological activity, areas in the cell, appearing optically as granules, are constantly appearing and disappearing and that the number demonstrable at any one time remains approximately constant.

The increase in the number of visible bodies may accordingly be due to the fixation of these structures by the dye, and they then persist. As new bodies are formed, they in turn become stained and rendered more or less permanent, and all or any of them may serve as nuclei about which more of the substance may be accumulated. That is, the concentration of dye in these areas may tend to change a reversible reaction to an irreversible one. Such a condition has been observed by Chambers ('14) in the staining of mitochondria with Janus green. He found that mitochondria when once stained are apparently unable to pass back into solution and, as fresh mitochondria are constantly forming, the number of stained mitochondria steadily increases.

Before discussing specifically the findings of the present paper, the relation of the form of the typical Golgi net, in fixed and impregnated cells, to the conditions which exist in the living cell should be considered. According to the concept of Parat, the Golgi substance is typically found in the form of vacuoles, the vacuome. Recent studies by Covell and Scott ('28) and Cowdry and Scott ('28) show that, at least in the tissues studied by them, the neutral-red granules and vacuoles may exhibit in the living state a definite alignment which is greatly accentuated by fixation and often accompanied by fusion. This material also exhibits a pronounced affinity for osmic acid. In other words, they have been able to observe the transformation of discrete neutral-red granules into the typical Golgi apparatus.

Other evidence of the essential granular, globular, or vacuolar nature of the Golgi apparatus is not lacking. Certain stains, such as trypan blue, exhibit a tendency to accumulate within living cells and in many cases appear as colored vacuoles or globules. The topographical relations of these groups of colored vacuoles simulate very closely the distribution of the typical Golgi apparatus (Chlopin and Chlopin, '25; Jasswoin, '25; Nassonov, '26, '27; and Glasunow, '28). Furthermore, Tretjakoff ('28), using both osmic-acid and silver-impregnation methods, has found that the Golgi substance, 'Liparosma Stoff,' in young, incompletely differentiated cartilage cells, appears as very small microsome-like granules. In many Protozoa, likewise, the Golgi substance exists as separate, distinct bodies.

Most workers apparently have approached a study of the Golgi apparatus with the preconceived idea that it must be of a reticulate pattern. This is strikingly shown in the case of Ludford ('27), who was stimulated to begin a study of the Golgi apparatus in the cells of tissue cultures by the results of Zweibaum and Elkner ('26 a, b), who succeeded in demonstrating the Golgi substance as a group of separate bodies by the method of da Fano. Ludford, with a different technic, was able to trace the stages by which a typically reticulate Golgi apparatus becomes changed into an atypical or disperse form. In the light of recent work, the stages might have been read in the reverse order. In many papers we also find statements regarding fragmentation, dispersal, or disintegration of the Golgi network. If the observations of Covell and Scott ('28) and Cowdry and Scott ('28) are found to hold generally for tissues, it is obvious that the degree of fusion of the separate Golgi elements will be influenced by such factors as the degree of their dispersal through the cell and the viscosity of the intervening cytoplasm. Indeed, it may also be true that, in cases where the Golgi elements are closely packed, a considerable degree of fusion may spontaneously occur.

The observations of Lewis ('24) on the appearance and behavior of the neutral-red granules and the centrosphere in mesenchyme cells in tissue culture is not without interest in a study dealing with blood cells. Lewis finds that normal mesenchyme cells contain very few or no neutral-red granules, or vacuoles, but they quickly appear under abnormal conditions in the culture. He regards them as evidence of degeneration and feels that their affinity for vital dyes is an accidental affair. They tend to become accumulated about the centrosphere, which slowly enlarges, and the mitochondria also take on a definite radial arrangement about this area. As degeneration proceeds, the centrosphere gradually enlarges, pushing granules, vacuoles, and mitochondria before it. The giant centrospheres usually present a clear medullary zone and a wide cortical zone. The medullary zone, when it first appears, is surrounded by a zone of fine granules; as the cortical zone develops, most of the granules seem to be pushed to its periphery, but a few usually remain for a while between the two zones. Lewis accordingly believes that the term segregation apparatus is a misnomer and that the whole apparatus is a phenomenon associated with degeneration.

A different view of this matter is taken by Key ('28) in his study of the cells of normal synovial fluid. He finds that, in the resting monocyte of the joint fluid, the neutral-red granules of the rosette are larger and less numerous than are those of the blood monocytes. Most of the cells also contain a few medium-sized neutral-red granules which lie in the peripheral zone of cytoplasm. He regards these cells as being in a state of mild stimulation. Clear unstained vacuoles may also appear in these stimulated monocytes.

In view of the many conflicting opinions regarding the neutral-red bodies, or the segregation apparatus of blood cells, and their possible relation to the Golgi apparatus, the interpretation of the present findings in the blood cells of Necturus presents obvious difficulties. In none of the non-hemoglobin-bearing elements is the situation as clear-cut as in the erythrocyte, which was dealt with previously. In the red blood cell the apparatus reacted with basophilic dyes in smears, could be readily distinguished in fresh blood, took up neutral red rapidly in supravital staining, and blackened typically with osmic acid in hanging-drop preparations or when impregnated by the method of Nassonov-Kolatchev.

In all the leucocytes in which the apparatus has been distinctly observed it is always associated with the centrosphere. No evidence of a persistence of this structure in the erythrocyte was obtained. In the thrombocytes the occasional occurrence of a vacuole-like area in the midst of the granular cytoplasm is suggestive. Jordan ('19) and Maximow ('28) derive the thrombocytes largely from lymphocytes, and Maximow has observed this transformation in cultures of chicken blood (figs. 160 to 168). In the early stages a small neutral-red rosette is common. Later it disappears and, instead, pale reddish droplets appear which are often located in the cytoplasm near the poles of the nucleus. He makes no mention of the centrosphere.

In the thrombocytes, lymphocytes, and monocytes the most convincing evidence is obtained that the neutral-red granules may be impregnated with osmic acid, and of these cells the lymphocytes exhibit the most constant pattern. In the lymphocytes the blackened granules, apparently under the influence of the centrosphere, exhibit either a concentric or radial pattern, with some evidence of fusion, but correspond closely to the pattern of the neutral-red granules.

In many monocytes and neutrophiles the pattern of the osmiophilic substance is varied. In some instances it appears to consist of enlarged and partially fused globules, at other times it is seen as a cluster of radially arranged rods or filaments. In size and shape these bodies resemble the bacillary chondriosomes (Karpova, '25[1]) which are frequently seen in lymphocytes. On the other hand, they may be interpreted as the products of the fusion of the radially aligned neutral-red granules. In some cases their beaded appearance is suggestive of such an origin; in others, the contour of the rod-like bodies is smooth. The segregation apparatus was not clearly seen in the eosinophiles, but the pattern of the osmiophilic substance exhibits the same variations as that of the monocyte and neutrophile. Nothing definite can be concluded regarding the basophiles.

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[1] According to Parat ('28, Arch. d'anat. micro., T. 24, p. 335), certain of the chondriosomes (chondriosomes actifs, lépidosomes) are specially oriented in the

To summarize, we may say that the evidence, that the segregation apparatus and the Golgi apparatus are identical structures, is by no means as direct for the cells under discussion as it is in case of the erythrocyte. However, there is rather strong circumstantial evidence that in the thrombocytes and leucocytes the structures are identical.

The variable and irregular pattern of the Golgi substance often encountered in the monocytes, neutrophiles, and eosinophiles may possibly be due to the greater specialization and activity of these cells, and the constancy in the form of the Golgi apparatus in the lymphocyte may accordingly be regarded as evidence of the less differentiated state of this cell. The distribution in the erythrocyte and thrombocyte may also be thought of as a visible expression of the bipolarity of these cells, acquired by metamorphosis from lymphocyte forms.

It is also in the erythrocyte, lymphocyte, and monocyte that there is found the best evidence for advancing the hypothesis that the so-called azurophilic granules are the bodies which store neutral red. In these cells the topography of the granules is quite definite and the similarity to the granules of the

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'zone of Golgi' and are frequently demonstrated by metallic-impregnation methods. In such preparations the vacuome often may not be impregnated. This variable reaction, he believes, explains Karpova's results which led her to conclude that dictyosomes were derived from chondriosomes, and were not identical with the vacuoles. In the blood cells of Necturus certain of the more filamentous bodies reacting with the osmic acid may possibly be interpreted as modified chondriosomes. Attempts to verify the presence of these modified elements in the region of the segregation apparatus with Janus green gave inconclusive results.

segregation apparatus in size and position is striking and suggestive. The granules in the region of the centrosphere of the neutrophiles, other than the specific granulation, are regarded by Jordan ('19) as essentially elements of the astral system, being derived from the unicorpuscular sphere (centrosome) by a process of partition or disintegration. Apparently no critical observations have been made of similar granules associated with the centrosphere of the eosinophiles.

It becomes increasingly obvious, therefore, that the question of the identity of the azurophile granules (possibly krinom substance), the neutral-red bodies, and the Golgi apparatus must remain in some doubt until more is learned about these several cell elements.

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## PLATE 1

### EXPLANATION OF FIGURES

All seven figures are microphotographs: Zeiss compensating ocular no. 8 and Zeiss $\frac{1}{12}$ oil-immersion objective. The absolute magnification, however, is not the same in all cases, owing to variations in the extension of the bellows of the camera. Photographs 1 and 4 are of living cells. Photographs 2, 3, 5, 6, and 7 are from fixed preparations—technic of Nassonov-Kolatchev.

1 A monocyte stained supravitally with neutral red for twenty-four hours after the method of Sabin ('23), showing the stained granules of the segregation apparatus grouped in the depression of the nucleus. This monocyte was rounded up and the granules were typically arranged about the area of the centrosphere when the preparation was placed under the camera, but the intense illumination caused the monocyte to resume ameboid activity, movement being in the region of the arrow. During the twenty-four hours the granules have enlarged and appear fewer in number, due possibly to coalescence. In this state they are more aptly designated as globules.

2 Two monocytes from the inferior vena cava; Nassonov-Kolatchev technic, five days at 35°C. The one on the left is out of focus, but the one on the right shows a typical rosette of blackened granules. They are less clumped in the preparation than they appear in the photograph. This cell resembles very closely the picture presented after twenty minutes' exposure to neutral red, applied supravitally.

3 An eosinophilic leucocyte lying in contact with the endothelium of a small blood vessel of the mesonephros; Nassonov-Kolatchev technic as in figure 2. The other blood cells are out of focus. In this cell the bilobed nucleus and the coarse, radially grouped, specific granules can be seen. In the region of the centrosphere (compare with fig. 15) a blackened mass is seen. It appears to be composed of globules connected by frequent coarse bridges. The entire mass, unfortunately, is not shown in this section.

4 A neutrophilic leucocyte stained supravitally with neutral red for twenty-six hours. As in figure 1, this cell was rounded up when placed under the camera, but when illuminated for photographing it sent out several filamentous pseudopodia in the region indicated by the arrow. The several lobes of the nucleus and the fine, specific granules are clearly shown. In the region of the centrosphere (fig. 14) a cluster of neutral-red globules and granules is present. As in the monocyte (fig. 1), many of the granules have increased in size and have assumed a globular

5 An eosinophilic leucocyte from a small blood vessel of the mesonephros, as in figure 3. The osmiophilic bodies in this case, however, are grouped and radially arranged in the region of the centrosphere. A few can also be seen scattered through the cytoplasm. They appear as short rods.

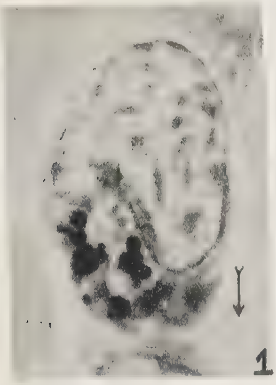
6 A neutrophilic leucocyte from the aorta; technic, Nassonov-Kolatchev. The nucleus is not shown in this section. The osmiophilic substance is grouped and exhibits a radial arrangement presumably about the region of the centrosphere. The blackened bodies consist of granules and slender rods. A few unblackened vacuoles are also associated with this structure.

7 A lymphocyte from a sinusoid of the mesonephros; technic, Nassonov Kolatchev. Directly opposite the cleft in the nucleus there is a cluster of small blackened granules arranged in a rosette and forming an inner and an outer circle. The granules composing each circle are joined to one another by delicate black filaments. In the cytoplasm on the opposite side of the nucleus numerous fine gray granules are present. They are probably mitochondria.

## SEGREGATION APPARATUS IN AMPHIBIAN BLOOD CELLS

PLATE 1

ALDEN B. DAWSON AND CHARLES SPARK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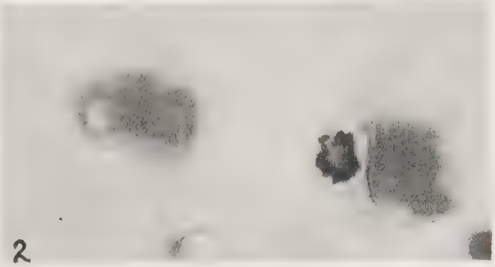
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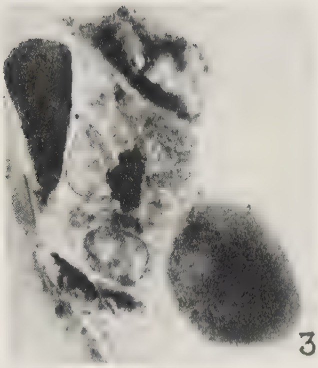
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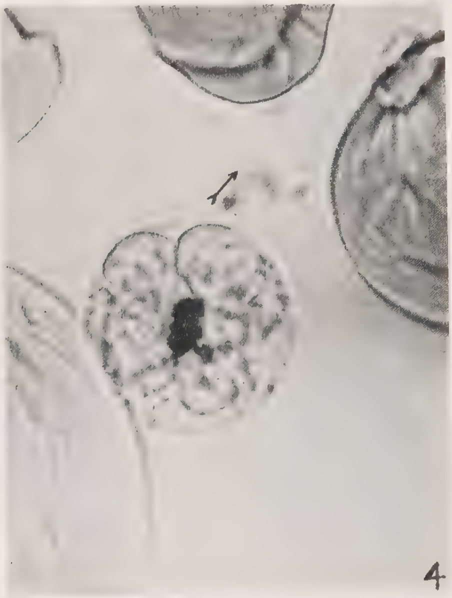
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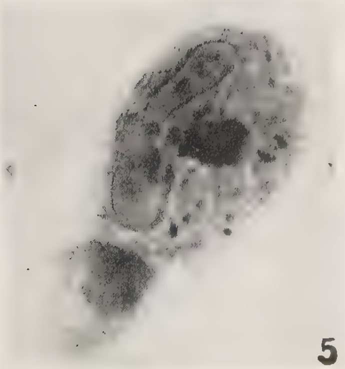
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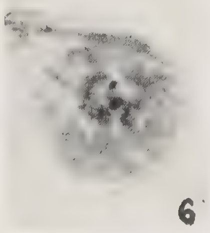
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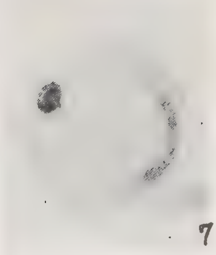
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# PLATE 2

## EXPLANATION OF FIGURES

The figures are drawings of fixed cells. The main features of the cells were outlined with a camera lucida and the details drawn in as accurately as possible.

8 This cell was not positively identified, but it is regarded as a small monocyte, and was found in the inferior vena cava; technic, Nassonov-Kolatchev. The osmiophile substance is seen as a group of discrete, blackened granules in the region of the indentation of the nucleus. In the cytoplasm outside of this region irregular gray granules, presumably mitochondria, are seen.

9 A monocyte from a mesonephric sinusoid; technic, Nassonov-Kolatchev. In this cell the osmiophilic substance is grouped, but does not exhibit a radial arrangement. It consists of irregular curved elements and probably separate granules.

10 and 11 Two thrombocytes from a hanging-drop preparation after exposure to the fumes of a 2 per cent solution of osmic acid for six days. In these cells the cytoplasm is largely concentrated at the two poles of the nucleus and exhibits a peripheral hyaline zone, and a more proximal granular zone nearer the nuclear membrane. In the granular zone several blackened granules are seen. The other granules in these regions are a dark gray. In figure 11 the granules at one pole are arranged in a manner suggestive of a rosette, but no conclusive evidence of the presence of a centrosphere was obtained by any technic used in this study.

12 Neutrophilic leucocyte stained with Wright's blood stain. Nucleus, light blue; cytoplasm and specific granulation, a light lilac; clusters of coarser granules, purple-red.

13 Neutrophilic leucocyte from a hanging-drop preparation exposed to the fumes of osmic acid for eight days. The osmiophilic substance is arranged as slender rods radiating from the region of the centrosphere.

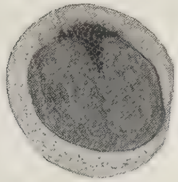
14 Neutrophilic leucocyte prepared as in figure 12. In this cell the coarse, densely stained granules are grouped in a single cluster and exhibit a tendency to be radially arranged.

15 An eosinophilic leucocyte stained with Wright's blood stain. Nucleus, light blue; specific granules, bright red. The finer granules in the region of the centrosphere are stained more lightly and have a slight basophilic tint.

16 A monocyte from a large blood vessel of the mesonephros; technic, Nassonov-Kolatchev. The osmiophilic substance is seen as clusters of clumped granules connected by narrow bridges. One cluster is in contact with a gray-colored vacuole.

17 and 18 Two large lymphocytes from a vessel of the mesonephros; technic, Nassonov-Kolatchev. The blackened granules exhibit some evidence of fusion and appear to have been arranged as a rosette with a definite radial alignment.

19 A monocyte (spheronuclear type of Jordan) from a film stained by Wright's method. Nucleus, light blue; cytoplasm, gray-blue. The basophilic reaction is more intense in a semicircular area about the centrosphere. In this area a variable number of purple-red granules, often exhibiting a radial alignment, are present.



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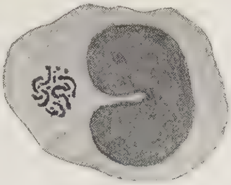

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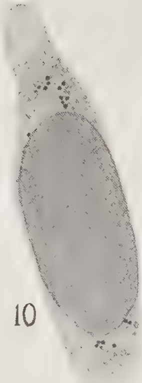

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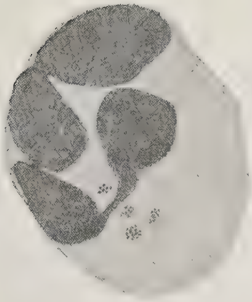

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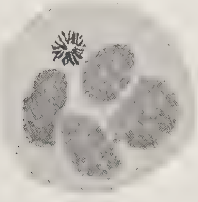

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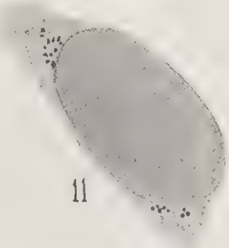

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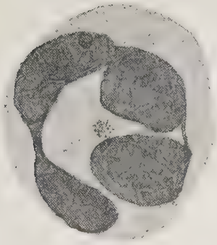

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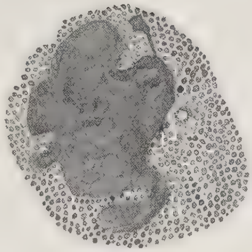

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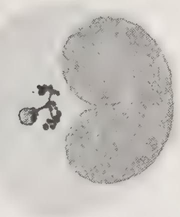

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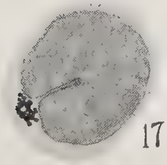

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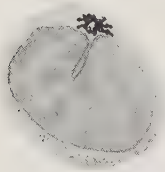

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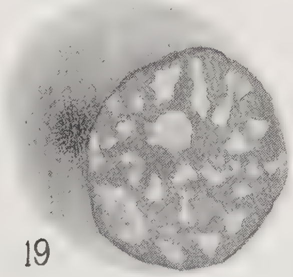

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# STUDIES OF HYPERTHYROIDISM

## V. OBSERVATIONS UPON THE POSTERIOR LYMPH HEARTS AND LYMPH VESSELS IN LIVING FROG LARVAE UNDER TREATMENT WITH THYROID EXTRACT

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TWO FIGURES

The posterior lymph hearts in frog tadpoles are located just above the hind limbs (fig. 1). With the aid of the upright chamber of Clark ('12), their rapid pulsations may be readily observed under the low power of the microscope. Their function in the tadpole is to facilitate the return of the lymph from the ponderous tail. After metamorphosis, they perform a similar function in returning the lymph from the hind limbs. The purpose of this study has been to find out by direct observation of the living animals whether excess thyroid autacoid, either directly or indirectly, affects the behavior of these lymph hearts and their tributary lymph vessels.

Tadpoles of the wood frog (Rana sylvatica) and of the tree frog (Hyla crucifer), collected at Woods Hole, Massachusetts, in June and July, were selected for observation. The wood-frog tadpoles had small hind limbs and usually possessed three pairs of posterior lymph hearts. Some, however, had four pairs (fig. 1) and some only two pairs. A few had three on one side and two on the other; a few others had three on one side and four on the other. The tree-frog tadpoles usually had one pair of posterior lymph hearts, though two pairs were also common. In a number of cases two lymph hearts were present on one side and only one on the other.

Although usually the hearts on the same side beat together, they were by no means perfectly synchronized. Pulsation counts in the tree-frog tadpoles were made from the most anterior heart; in wood-frog tadpoles, from the heart next to the most anterior one. Since the pulsation rate varied within wide limits, an average of from three to six counts was always taken for the daily observation upon each animal. No anesthetic of any kind was used. The hyperthyroid condition was induced by the addition of desiccated thyroid extract to the water in which the animals were kept.

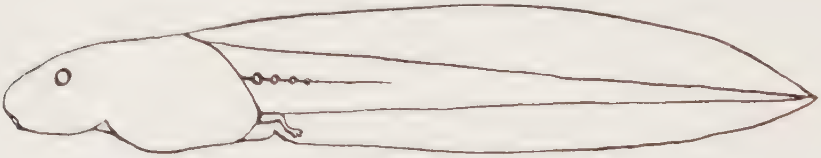

Fig. 1 Tadpole of the wood frog (Rana sylvatica). The location of the posterior lymph hearts is indicated by the four circles along the lateral line near the root of the tail.

## OBSERVATIONS

Table 1 gives a summary of the observations made upon normal and hyperthyroid tadpoles of both the wood frog and tree frog. It will be noted that, in the first species, four days

TABLE 1

| | *Average number of lymph-heart beats per minute* |
|---|---|
| Rana sylvatica (wood frog) tadpoles: | |
| 24 normal tadpoles (two limbs), | 144 |
| 24 thyroid-treated tadpoles (two limbs before treatment) after four days, | 92 |
| 10 tadpoles undergoing spontaneous metamorphosis (four limbs and tail in various stages of resorption), | 74 |
| 8 completely metamorphosed tadpoles during the first week following metamorphosis, | 107 |
| 3 completely metamorphosed tadpoles, three weeks after metamorphosis, | 116 |
| 4 completely metamorphosed tadpoles, one month after metamorphosis, | 125 |
| Hyla crucifer (tree frog) tadpoles: | |
| 64 normal tadpoles (two limbs), | 142 |
| 61 thyroid-treated tadpoles (two limbs before treatment) after three to four days, | 96 |

of thyroid administration brought about a fall of fifty-two beats per minute, i.e., a decrease of 36 per cent; and in the second species, a fall of forty-six, or a decrease of 32 per cent.

But this fall was not immediate, as evidenced by the daily individual records of many of these animals. During the first two days little significant change in pulsation rate could be detected. While some animals showed an increase, others showed a decrease, and still others remained about the same. During this period there were no conspicuous macroscopic changes in the tail and hind limbs. Beginning with the third day in tree-frog tadpoles and slightly later in the wood-frog tadpoles, there occurred a definite decrease in rate of lymph-heart pulsation. This reduction was even more pronounced at the end of four and five days. It was also during this period that the tail exhibited rapid resorption, with a marked fall in the amount of blood circulating in the tail. Growth of the hind limbs also progressed rapidly. Metamorphic stasis usually followed, ending in death after five to seven days from the time of the first thyroid treatment. In larvae that were relatively mature at the start of the experiment metamorphosis was often successfully accomplished.

Included in table 1 are some observations upon wood-frog tadpoles in various stages of normal spontaneous metamorphosis. Ten individuals with well-developed fore and hind limbs, and tails in various stages of resorption, exhibited an average lymph-heart pulsation of 74. Great variation was noted in these, both in the different individuals and in the same individuals at different times. Somewhat later during the first week of metamorphosis, an average beat of 107 was found (eight animals). After three weeks of metamorphosis, the rate of pulsation was 116 (three animals); and after one month of metamorphosis, the average rate of pulsation was 125 (four animals).

There is no evidence in these data to show that the thyroid hormone has any direct and specific effect on the pulsation of the posterior lymph hearts. Indirectly, however, it brings about a marked decrease in rate of pulsation by causing tail involution with concomitant reduction of blood and lymph circulation in this region. This effect is displayed both by animals undergoing spontaneous metamorphosis and by those undergoing thyroid-accelerated metamorphosis.

In a number of tadpoles involution of the lymphatic vessels in the transparent tail fin was observed from day to day under the high power of the microscope. In a typical tree-frog tadpole, within twenty-four hours after thyroid treatment was started, the lymphatic endothelium in the transparent tail fin became somewhat vague and granular in places, especially in the smaller vessels. Some of the endothelial nuclear areas had disappeared and retraction of some of the smaller vessels had begun (fig. 2, b). After forty-eight hours, the wall of the vessels had become thick and the lumen narrow. More of the nuclear areas had vanished (fig. 2, c). After seventy-two hours, there were signs of marked degeneration. The vessel walls were irregularly thickened, and granules and vacuoles were conspicuous (fig. 2, d). The endothelial nuclei were vague and indistinct. Throughout the entire tail fin, retraction and resorption of lymphatics continued progressively, starting with the smaller vessels and proceeding to the larger.

Fig. 2 a. Lymph vessels from the dorsal fin expansion near the middle of the tail of a tadpole of the tree frog (Hyla crucifer). The positions of the nuclear areas of the lymphatic endothelium are shown. This sketch was made immediately before the tadpole was subjected to thyroid treatment. The arrows indicate the direction of the tip of the tail and the dorsal edge of the fin expansion.

b. The same lymph vessels, twenty-four hours after thyroid treatment. Some of the endothelial nuclei are gone, and in places the walls are somewhat granular. The distal portion of the lower lymphatic shows retraction and ends blindly. Two polymorphonuclear leucocytes may be seen along the former line of the vessel (at the lower right of the figure).

c. The same lymph vessels, forty-eight hours after thyroid treatment. Regressive changes are apparent in the lower and smaller vessel and are beginning in the upper vessel. Some endothelial nuclear areas have disappeared, and some have become granular and vague in outline.

d. The same lymph vessels, seventy-two hours after thyroid treatment. A dilated blind pouch is present at the right where the upper vessel formerly branched into two vessels. Degenerative changes are marked throughout.

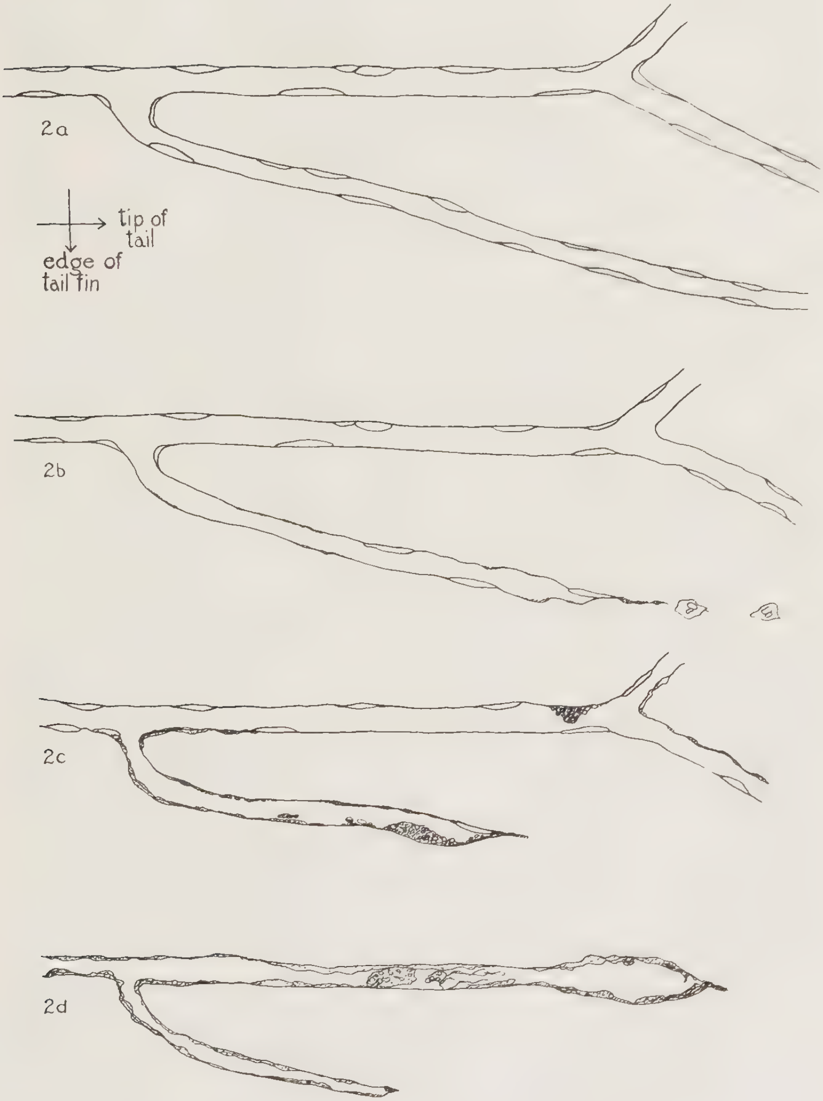
2a
tip of tail
edge of tail fin
2b
2c
2d

Figure 2

Clark ('12) has followed in some detail the movements of the nuclear areas of lymphatic endothelium under normal conditions. He points out that during development a small lymph vessel occasionally becomes superfluous and undergoes retraction. This retraction is preceded by the retreat of the nuclear areas into the parent stem. Evidently, in the thyroid-treated tadpoles of my experiments, a similar migration of endothelial nuclear areas occurs to a marked degree, which foreshadows the approaching processes of retraction and resorption.

In the blood vessels similar changes occurred, though not so rapidly. Stasis appeared first in the smaller capillaries and then in the larger branches. Retraction of capillaries often followed. Sometimes disjunction took place, leaving short capillary fragments full of blood separated from the general circulation. Blood stasis in small capillaries was present after twenty-four hours of thyroid treatment. Contraction and retraction of blood capillaries were seen after forty-eight hours.

It was noted that the involution of small lymphatic vessels and of blood vessels in the tail took place before the lymph hearts showed any significant deviation from the normal pulsation rate. This suggested that the reduction of lymph-heart pulsation rate was a direct result of the reduced amount of lymph and blood that circulated in the tail after thyroid treatment, and not due to any direct and specific effect on the lymph hearts themselves.

Observations upon older animals showed that the average pulsation rate rises again after metamorphosis is completed. This is thought to be due to the rapid increase in lymph and blood circulation accompanying the growth and development of the hind limbs and to the adjustment of the lymph hearts to their new principal function of aiding the return of the lymph from the hind limbs.

A few other incidental observations are of interest. Often the lymph hearts ceased beating or beat irregularly during defecation. Sometimes, for a few seconds immediately preceding violent muscular exertion, the pulsations ceased.

In extreme hyperthyroidism the beats became somewhat irregular. There was often complete cessation of the pulsation of the most posterior lymph heart; that is, the fourth or third heart in wood-frog tadpoles and the second heart in tree-frog tadpoles.

After complete metamorphosis in wood-frog tadpoles, the hearts progressively became more closely crowded together at the stump of the resorbed tail. The beats became more synchronized on the same side. In at least one completely metamorphosed specimen all three hearts could be distinguished beating; in most of the others, however, only one or two could be distinguished.

With the approach of death to animals in metamorphic stasis, some of the hearts ceased beating. The fourth one was the first to stop, then the third.

In three cases in wood-frog tadpoles it was observed that at the beginning of the thyroid administration there were only three pulsating posterior lymph hearts on one side, but that after a day or two a fourth heart started beating. In tree-frog tadpoles one case was noted in which a second heart started beating under thyroid treatment. In the later stages of thyroid treatment these ceased pulsating. It is, of course, possible that these hearts may have started beating if the thyroid extract had not been administered. In any case, the presence of the thyroid hormone did not inhibit the development of a differentiation that is a purely larval character, and it may actually have stimulated this differentiation. The general truth of this principle is amply upheld by the results of experiments dealing with the effects of thyroid treatment on regenerating tissues of different ages (Speidel, '29).

Complete cessation of lymph-heart beat for longer than a half minute is quite rare, except after extreme hyperthyroidism or after tail resorption has become marked. One stop as long as two minutes and one as long as seventy-five seconds were noted. After complete resorption of the tail, there is a reduction of 'flutter beats.'

## SUMMARY

Following administration of thyroid extract to frog tadpoles, the posterior lymph hearts exhibit no significant variation in rate of pulsation during the first two days. After that a marked reduction occurs. Average counts from twenty-four normal and twenty-four experimental wood-frog tadpoles show that, after three to five days of thyroid treatment, the pulsation rate is reduced from 144 beats per minute to 92—a reduction of 36 per cent. Average counts from sixty-four normal and sixty-one experimental tree-frog tadpoles show that, after three to four days of thyroid treatment, the pulsation rate is reduced from 142 to 96—a reduction of 32 per cent. At the same time there is an increase in rate of blood-heart beat and also in respiratory rate.

The evidence indicates that thyroid hormone has no specific direct effect upon the lymph-heart pulsation rate. It does, however, accelerate retraction and degeneration of the smaller lymph vessels. This may be seen sometimes after one day of thyroid administration, and becomes quite conspicuous after two or three days. Migration of some of the endothelial nuclear areas from the smaller to the larger vessels precedes the process of degeneration. General tail resorption follows. The fall in lymph-heart pulsation rate, therefore, is regarded as a result of the resorption of the tail together with the associated decrease in the tail lymph circulation. The later recovery in lymph-heart pulsation rate that occurs after complete metamorphosis is probably influenced by the increased lymph circulation in the growing hind limb.

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# A NOTE ON THE SPERMATOGENETIC WAVE IN THE TESTES OF THE ADULT ENGLISH SPARROW (PASSER DOMESTICUS)

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In 1921, the writer began an investigation of the spermatogenesis of the English sparrow. This problem was begun in the Zoölogical Laboratories of the University of Wisconsin at the suggestion of Dr. M. F. Guyer. The purpose of the investigation was twofold: 1) the investigation of the cycle of spermatogenesis, and, 2) to determine, if possible, whether there was any correlation between the sex-chromosome condition as found in a pure species, such as the sparrow, and that found in such a hybridized species as the common fowl.

Material was collected at various times, the hours of collection ranging from 1 P.M. until 5 P.M., during the season when the testes were actively producing spermatozoa. The collections were made during the months of February, March, April, May, and June. In all the material collected the investigator was unable to find a single mitotic figure in which the chromosomes were formed. Another student in the same laboratory, Neva Helen Drummond (Neva Drummond Foley), collected a large amount of material over approximately the same monthly periods and within about the same daily time limits, and likewise secured uniformly negative results. The joint investigations extended over a number of years, from 1921 through 1926.

This apparent lack of stages later than resting stages of the spermatogonial or the pachytene stage of the first maturation division led the writer to believe that possibly there existed a daily cyclic rhythm of division in the seminiferous tubules. Hance ('25) is of the opinion that there are indications of spermatogenesis going forward in waves in the testes of the sparrow. In the spring of 1928, the investigation was resumed in the Laboratories of Anatomy at Tulane University. Material was collected from 7.37 until 12 M. during the first three weeks of March and from 5.30 A.M. until 12 M. throughout the months of April, May, and June. All the material secured, during the period embraced by the years 1921 to 1926, was collected at such time intervals so as to include specimens representing a sampling of at least each hour from 1.00 to 5.00 P.M. Since that failed to show sufficient of the stages, it was only deemed necessary at this time to collect over the forenoon periods. However, to be reasonably sure that such a cycle does not exist during the afternoon hours, material is now being collected at twenty- to thirty-minute intervals during each hour of the afternoon.

One of the difficulties encountered by students of avian spermatogenesis, and in particular by those working on the adult testis, has been the scarcity of the desired stages in the mitotic process. Although points have arisen which necessitate further work on the problem, the writer feels that data have been obtained which indicate rather definitely the existence of a periodicity in the cycle of mitosis in the tubules of the sparrow's testes. The results set forth in this article are deemed to be of interest to workers in this field, in that it may be found that daily cycles of division occur in the testes of other birds with which this difficulty has been experienced.

Throughout the month of January and the majority of February the testes show only spermatogonia. Early phases of the first maturation division and transforming spermatids are to be found during the last week in February. By the first week in March, the cycle of spermatogenesis is proceeding apace. The time marking the onset of maturation has been verified in the specimens collected over a number of years. Since no daily cyclic rhythm had been noted in the material collected previously during the afternoon, it was thought that, if a rhythm of division takes place in the testes of the sparrow, collections made at frequent intervals during the forenoon would disclose it. It was thought that these collections should begin to be made as early as possible in the morning and certainly on till 12 M. Material was collected, at intervals of ten to thirteen minutes, from 7.37 A.M. to 12 M. from the 3rd to the 25th of March. Material collected at 7.37 showed all the mitotic phases of the first and second maturation divisions. By 7.50 few of the later phases are to be found, and by 8.00 A.M. the testes show only the early phases of the first maturation division. Throughout the remainder of the morning, up until 12 o'clock, during these first three weeks of March, stages later than the pachytene of the meiotic prophase cannot be found in the seminiferous tubules.

As stated above, material previously collected in the afternoon during April, May, and June had shown only resting spermatogonia, early prophases of the first maturation division, and transforming spermatids. The presence of early stages of the first maturation division indicated that the cycle was being completed at some time later during the twenty-four hours. Supposing that the mitotic phases of the first and second maturation divisions have the time limit in the daily cycle of from 7.37 to 7.50 A.M., as shown in the material collected in March, it was reasonable to suppose that the same time limits would be found for these later months. However, material collected during the forenoon at frequent intervals from 7 until 12 o'clock, during April, May, and June, failed to show the later phases of mitosis as shown by the March material. Then it was thought that the cycle might be shifted to an earlier hour during these months. With the determination of such a possibility in view, the hours of collection were advanced to 5.30 A.M. and material has been obtained from this hour at approximately every twenty to thirty minutes throughout the morning hours up to 12 o'clock, and these collections have failed to show any division stages other than early prophases of the first maturation division.

However, this does not exclude the possibility of a cycle occurring before 5.30 and makes necessary collections during the hours of the night between 6.00 P.M. and 5.30 A.M.

The existence of daily rhythmic cycles in plant mitosis is well established (Sharp, '26), but this problem has received only slight attention by animal cytologists. The only piece of work on the periodicity of the maturation cycle in birds with which the writer is familiar is that by Harper ('04) on the pigeon ovum. Harper states that the first maturation division in the pigeon ovum occurs between 7.40 and 9.00 P.M., the second between 7.45 and 10.15 P.M. It is suggestive that the maturation divisions in the pigeon take place late in the day. Cycles may occur after 6.00 P.M. in the sparrow. The material collection of which is now under way should uncover any such existing cycles in the sparrow. Spermatogonial division figures have not as yet been observed, and it is entirely possible that cycles of division will also be found, between 6.00 P.M. and 5.30 A.M., for this stage of the spermatogenetic cycle. The writer has had to have recourse to early embryonic testes for spermatogonial mitosis.

The spermatogenetic wave may possibly be correlated with the physiological state of the bird. During the early part of March the birds begin to fly about and assume their usual daily physical activity around 7.00 A.M., while throughout the later months the period of activity is advanced to the neighborhood of 4.00 A.M. Collections have not as yet been made earlier than 5.30 A.M. During the greater part of the month of March the birds are not as active from 7.00 to 8.00 A.M. as they are later in the day. The fact that complete mitosis was found only in those testes collected during March, between 7.00 and 8.00 A.M., would lend credence to the idea that there is a correlation between the state of physical activity of the bird and the completion of mitosis. In other words, mitosis appears to go to completion during the interval between the beginning of the rest period (after 6.00 P.M.) and the beginning of maximum activity. Certainly it is significant that the later phases of mitosis are not found throughout the remainder of the day, during which the birds are very active, although the earlier phases of the first maturation division and transforming spermatids exist in abundance. Of course, the possibility exists that the later phases of division take place during maximum activity, but very rapidly, and they are not caught within the periods during which the material has been collected. If the cycle is correlated with the time interval between beginning of rest and beginning of maximum activity, then additional cycles may be demonstrated for months, other than March, during which the period of pronounced physical activity is shifted to earlier hours of the day. This would explain the failure to find complete mitosis between 7.00 and 8.00 A.M. during the latter part of March and the succeeding spring and summer months.

The chromosomes of the sparrow are quite small, and it is questionable if the sparrow will prove a fertile field for work on avian chromosomes. As the various points in the spermatogenetic cycle will be discussed in a separate paper, it need only be stated here that the somatic number of chromosomes is around forty.

In conclusion, it may be stated that there is positive evidence of a periodic rhythmic cycle of cell division taking place in the testes of the sparrow. Although more than one cyclic wave may occur, only one cycle has been positively located to date. In this cycle the first and second maturation divisions take place between 7.00 and 8.00 A.M. during the first three weeks of March. Specifically, complete first and second maturation divisions were found to occur during this month from 7.37 to 7.50 A.M. There are indications that the rhythmic waves of division are correlated with the degree of activity of the bird: that they occur between the beginning of the rest period and the beginning of pronounced physical activity.

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# A NOTE ON THE ORIGIN AND HISTOGENESIS OF THE MESONEPHRIC DUCT IN MAMMALS

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THREE FIGURES

The histogenesis of the primary excretory duct (the pronephric, mesonephric, or wolffian duct) has been a question of controversy. The majority of authors (Waldeyer, '70; Romiti, '73; Kowalewsky, '75; Koelliker, '76; Renson, '83; Meyer, '90, and many others) agree that the mesonephric or wolffian duct is derived from the mesoderm. According to Romiti ('73), the duct originates in chick embryos as an evagination of the pleuroperitoneal cavity. This evagination subsequently is cut off from this cavity and is constricted into a duct. Kowalewsky ('75) derives the duct from an evagination of the dorsal lamina of the intermediate cell mass, the latter being provided with a cavity communicating with the coelom and the somitic cavity. According to Gasser ('77) and Sedgwick ('81), the cephalic portion of the mesonephric duct of chick embryos develops from the intermediate cell mass and then grows freely caudalward, without having any direct connection with the intermediate cell mass below its point of origin. These authors, as well as Waldeyer ('70), Balfour ('81), and Mihalkovicz ('85), in contradistinction to Romiti and Kowalewsky, considered the duct as an originally solid rod of cells, which only later becomes provided with a lumen. His ('65) at first regarded the mesonephric duct of the chick as derived from a thickened fold of the ectoderm, by the side of the neural fold. Later ('68), His withdrew this statement, which, however, has been adopted by Hensen

('67) and Graf Spee ('83) for mammals. Graf Spee considered that the anlage of the mesonephric duct lay in that part of the ectoderm which is opposite the intermediate cell mass. Here, according to this author, the ectoderm forms in the caudal portion of the embryo a definite thickening of limited extent. This thickening was thought to separate cephalad into the ectoderm proper and the anlage of the mesonephric duct. Still farther cephalad, the duct was thought to separate entirely from the ectoderm and to enter into close contact with the intermediate cell mass. According to Hertwig ('10) and Meyer ('90), the cephalic portion of the primary excretory duct develops from the mesoderm. Caudalward, the duct separates from its parent tissue and fuses with the ectoderm. In Meyer's human embryo (of 4.25 mm.) the duct in its most caudal portion is again free from a connection with the ectoderm. According to Hertwig, there is a possibility that in mammals the ectoderm may contribute to the development of the caudal portion of the duct. Felix ('04) believed that in mammals a longitudinal growth of the duct occurs, in which the ectoderm participates. However, no participation of the ectoderm in the formation of the mesonephric duct is admitted by Hertwig and Felix in bony fishes.

The studies of MacCallum ('02) and Gage ('05) have shown that the excretory duct consists, in human embryos at early stages of development, of a frontal and a caudal portion, which are separated from each other by a marked interval. These authors are inclined to consider the frontal portion of the duct as related to the homologue of the pronephros of lower forms, since its anterior extremity opens directly into the coelom, being ''evidently a direct turning in of the lining of the coelom'' (MacCallum). In the embryo of MacCallum the frontal portion of the excretory duct lies opposite the sixth to the ninth myotomes, while the caudal portion begins at the level of the tenth myotome and extends caudad to the level of the last (nineteenth) myotome. In the Gage embryo the presumed pronephric tubule lies at the level of the eleventh myotome.

Our own observations pertain to a study of a series of mammalian embryos from the Embryological Collection of the Department of Anatomy, University of Michigan, and particularly of series of guinea-pig and rabbit embryos of early stages of development. Their study has led to the general conclusion that the mesonephric duct is histogenetically derived from the intermediate cell mass of the mesoderm and acquires only secondarily in its caudal portion a close contact with the ectoderm. Three especially pertinent series are here particularly referred to: two guinea-pig and one rabbit series, since they afforded critical and favorable material bearing on the problem under consideration. Abundant other series, here not especially referred to, of preceding and succeeding stages were available.

In a guinea-pig embryo removed from the uterus fourteen days sixteen hours after insemination, in which fourteen somites are distinguishable, we can observe at the level of the twelfth to the fourteenth somites a more or less clearly delimited rod of cells lying dorsal to the intermediate cell mass. This cell rod is without lumen and is regarded as the anlage of the mesonephric duct. It is fused definitely in its frontal portion with the intermediate cell mass, while its narrower caudal portion enters into a close relationship with the ectoderm. In this embryo there is no trace of any structures which might be considered as the homologue of the pronephric tubules.

The embryo, after careful fixation, was cut serially in cross-sections of 7 μ thickness, stained in hematoxylin and Congo red. The series embraces 635 sections, numbering from the frontal pole of the embryonic shield to the caudal limit of the allantois. Of the fourteen somites determined, neither the first nor the last somite is provided with a cavity, such as is present in the other somites. The neural groove is found closed and forms a tubular structure from the level of the 32nd to the level of the 394th section, i.e., corresponding in general to the level at which the somitic segmentation is no longer evident. In certain sections the ganglion of the fifth nerve is cut off from the surface ectoderm and the neural groove, and lies lateral to the latter. The notochord in its most frontal part is in close contact with the entoderm of the foregut. It becomes entirely separated from the entoderm at the level of the second somite. It lies then in close relation to the neural tube ventrally. The first somite on the one side makes its appearance in the 117th section and on the other side in the 134th section.

The intermediate cell mass becomes distinguishable at the level of the sixth somite. The medial half of the dorsal aorta lies, at this level, in close relation to the ventral portion of the somite. Just lateral to the aorta lies the coelomic cavity, which is narrow in the mediolateral and larger in the dorsoventral plane and extends thus dorsalward and ventralward to the level of the aorta. The intermediate cell mass is often continuous with the somites medially and with the wall of the coelomic cavity laterally. The nuclei of the intermediate cells are, in general, more round than those of the cells of the coelomic wall. They are of a more distinct outline than are the nuclei of either the coelomic or the somitic mesodermal cells and the granulations are sharper in the former than in both the latter. Mitotic figures are frequently met with in the intermediate cell mass. In the region under consideration, the ectoderm forms a relatively thin, outwardly concave fold opposite the intermediate cell mass. It appears here slightly thickened. The apparent thickening is probably due, in part at least, to the orientation of the longer axis of the ectodermal cells to the plane of the section. Sometimes the nuclei of the ectodermal cells forming the fold are not arranged in a perfectly regular row. At the level of the sixth and seventh somites the intermediate cell mass is very small. It disappears almost entirely at the intervals between the somites. At the level of the eighth somite the intermediate cell mass becomes more distinct, extending from the coelomic to the somitic mesoderm, in close relation dorsally to the lateral half of the aorta. In cross-sections it appears large in its middle portion, while the cell bridges, which connect it with the somite medially and the coelomic wall laterally, are usually relatively narrow. The large middle part of the intermediate cell mass extends dorsally toward the ectoderm; however, it does not come in contact with the latter. Approximately at the level of the middle of the eighth somite the long axes of the cells of the intermediate cell mass on the one side are arranged radially around the center of the cell mass; in the center a small cavity is to be noted. The cell nuclei are situated at the periphery of the cells, at the pole opposite to the center of the cell mass. At the level of the caudal end of the eighth somite the intermediate cell mass becomes small, but it does not disappear entirely even at the place opposite the interval between the somites. The intermediate cell mass is again more pronounced at the beginning of the ninth somite, but only for a few sections, after which it again becomes smaller. At the level of the ninth somite the intermediate cell mass is narrower in both the dorsoventral and the mediolateral planes than at the level of the eighth somite. At the level of the tenth somite the intermediate cell mass appears as a quite dense band of cells between the somite and the coelomic wall and continuous with both of these.

At the level of the caudal portion of the tenth somite a bud is given off from the intermediate cell mass dorsalward, while this latter mass is, as before, connected with the coelomic and somitic mesoderm, chiefly with the former. At the level of the eleventh somite, this dorsal bud closely approaches the ectoderm. In cross-sections the bud presents a somewhat quadrangular form. The ventromedial side of the quadrangle is continuous with the intermediate cell mass, the dorsolateral side is parallel to the ectoderm and lies, as mentioned before, quite close to the latter. A shallow fold in the ectoderm at this level has its concavity directed inward, while in more frontal regions the concavity of the fold at relatively the same region is directed outward. The ectoderm is somewhat flattened in the region where the bud of the intermediate cell mass comes into contact with it. In some sections the whole intermediate cell mass has the form of a quadrangle, which is extended from the aorta to the ectoderm and which is connected on both sides with the coelomic and somitic mesoderm by small cell bridges. At the level of the twelfth somite the dorsal bud of the intermediate cell mass takes on a more nearly rounded outline (fig. 1, right side).

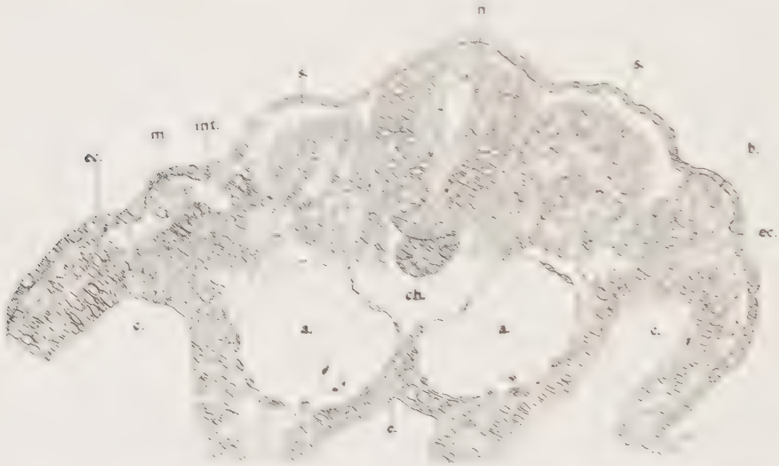

Fig. 1 Cross-section through the level of the twelfth somite of a guinea-pig embryo of fourteen somites. Camera-lucida drawing. × 182. The right side of the figure is slightly more frontal (middle of the twelfth somite) than the left (caudal portion of the twelfth somite). On the right side the intermediate cell mass gives off a bud (*b.*), which lies close to the ectoderm. On the left side this bud is already well delimited from the intermediate cell mass and recognizable as the anlage of the mesonephric duct. *a.*, aorta; *b.*, bud of the intermediate cell mass continuing caudad into the mesonephric duct; *c.*, coelom; *ch.*, notochord; *e.*, entoderm; *ec.*, ectoderm; *int.*, intermediate cell mass; *m.*, mesonephric duct; *n.*, neural tube; *s.*, somite.

It becomes more clearly delimited from the rest of the intermediate cell mass, though it is still, in the main, connected with the latter on the ventromedial side. The dorsolateral side is here in a still closer contact with the ectoderm. The intermediate cell mass, as before, is stretched out between the coelomic and somitic mesoderm. The bud differs from the rest of the intermediate cell mass by reason of its greater compactness. In general, its cells stain somewhat paler than those of the latter. The cells of the bud differ from the ectodermal elements in their staining reaction as well as in the shape of their nuclei. The nuclei of the ectodermal cells are often more elongated, their long axes being directed in a transverse plane, while the cell nuclei of the bud are, in general, more nearly round and with a somewhat sharper outline. Farther caudalward, but still at the level of the twelfth somite, the bud assumes a somewhat oval shape on one side. Its long axis is oriented in a transverse plane, the medial pole being more dorsal in position than the lateral. On the other side the bud remains more nearly round. In certain sections it is now quite clearly delimited from the rest of the intermediate cell mass. In some places only a small part of its ventromedial surface is continuous with the latter (fig. 1, left side). The nuclei of the intermediate cell mass are more elongated in this region, which is related to the coelomic mesoderm. Mitotic figures are often seen in the intermediate cell mass adjacent to the round or oval bud. The ectoderm forms, as before, an inwardly concave fold over the dorsolateral surface of the bud and lies in close contact with this surface. In certain sections through the level of the twelfth somite the bud of the intermediate cell mass is already recognizable as the anlage of the mesonephric duct, differentiating from the intermediate cell mass; it becomes entirely separated from this mass at the level of the thirteenth somite. The bud is smaller in size than the bud of the intermediate cell mass, of which it represents thus the caudal continuation. It is composed of from six to eight cells arranged in a round or oval mass without lumen and lying in close contact with the ectoderm dorsolaterally and with the intermediate cell mass ventromedially. The nuclei and the protoplasm of the cells composing the mesonephric duct, like those of the cells of the more frontally situated bud of the intermediate cell mass, appear somewhat paler than the elements of the intermediate cell mass. Their staining reaction is to a certain degree different from that of the ectodermal cells—in hematoxylin-Congo red preparations they present a somewhat more pink color than the latter, which are of a slightly more grayish tone. The nuclei of the cells of the mesonephric duct are nearly round, in contradistinction to the more elongated nuclei of the ectodermal cells. Farther caudalward the mesonephric duct becomes smaller and smaller. It is more fused with the ectoderm near the level at which the delimitation of the thirteenth somite from the fourteenth somite is indicated. At the level of the frontal portion of the fourteenth somite the mesonephric duct is represented in each cross-section by only two or three cells, which adhere to the ectoderm. These cells are here entirely separated from the ventromedially lying intermediate cell mass. In their staining reaction they approach more nearly the mesodermal than the ectodermal elements. In general, also, the juxtaposed cell boundaries between the ectoderm and the mesonephric duct are more distinct than those between the elements of the duct itself or of the ectoderm. In certain sections the mesonephric duct is still composed of more than two or three cells (fig. 2). In such sections these are sometimes arranged into a

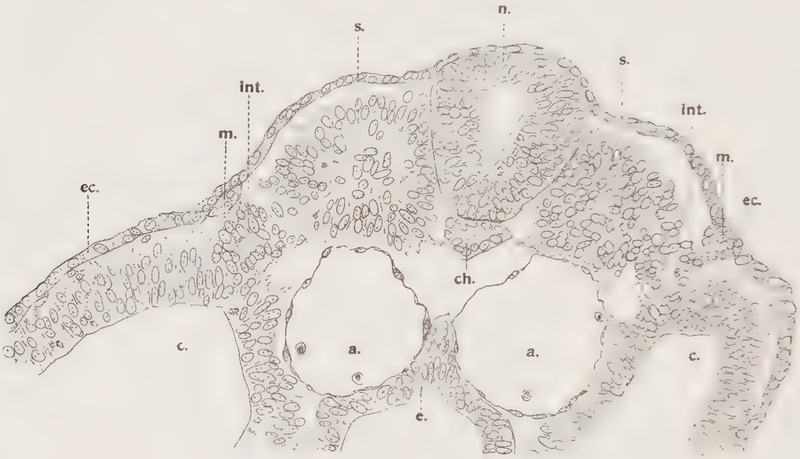

Fig. 2 Cross-section through the level of the fourteenth somite of the same embryo from which figure 1 was drawn. The right side of the figure is farther frontal than the left. Camera-lucida drawing. × 182. *a.*, aorta; *c.*, coelom; *ch.*, notochord; *e.*, entoderm; *ec.*, ectoderm; *int.*, intermediate cell mass; *m.*, mesonephric duct; *n.*, neural tube; *s.*, somite.

very short band situated parallel and close to the ectoderm. The duct disappears entirely at the level of the fourteenth somite. Until its caudal limit is reached, it is in very close contact with the ectodermal elements. The intermediate cell mass is here present as a short strip between the coelomic and somitic mesoderm, dorsal to the aorta. Caudalward the intermediate mesoderm becomes undifferentiable.

Thus, even in its most caudal part the mesonephric duct is distinguishable from the ectoderm by the different staining reaction of its elements and by a usually more or less distinct line of delimitation between both structures, which line is indicated by their cell boundaries. Such a differentiation can be made in spite of the fact that an occasional overlapping (fig. 2, right side), dependent on the plane of the section, might sometimes simulate a closer fusion than is actually present. The ectoderm forms a fold and sometimes a certain thickening at the place which corresponds to the intermediate cell mass. However, the thickening is more pronounced in regions where the duct is not yet differentiable from the intermediate cell mass. In more caudal regions, where the duct lies closer to the ectoderm, the latter is rather flattened at the place of contact.

In another guinea-pig embryo taken fourteen days sixteen and one-half hours after insemination, but of an earlier stage of development (eleven somites), the mesonephric duct is more or less delimited from the intermediate cell mass, but only for a relatively few sections. This embryo, after careful fixation, was cut in nearly transverse sections of 7 μ thickness and stained with hematoxylin and Congo red. The series includes 511 sections from the frontal end of the embryonic shield to the caudal end of the allantois. The intermediate cell mass, for the greater part, is continuous with the coelomic mesoderm and the somites, as in the embryo previously noted. At the level of the eighth to the eleventh somites the intermediate cell mass forms a dorsal bud of a round or sometimes more nearly quadrangular shape. This bud enters into close contact with the ectoderm. At the level of the frontal end of the bud the coelomic wall forms a shallow groove at the place which corresponds to the intermediate cell mass. However, the groove is present for only a very short distance. The bud of the intermediate cell mass represents only in a few occasional sections a more compact round or oval cell mass without lumen, which is more or less distinctly delimited from the intermediate cell mass. In sections where this occurs it can be identified as the anlage of the mesonephric duct. Its caudal and frontal limit continues into the bud of the intermediate cell mass. The ectoderm forms, as in the former embryo, a shallow fold at some places over the intermediate cell mass. Occasionally it appears thicker in these places.

In our series of early-stage rabbit embryos the origin of the mesonephric duct from the mesoderm and its primary independence of the ectoderm are also very evident. The description of the anlage of the duct in a rabbit embryo of fourteen somites follows. The embryo was fixed in Bouin's fluid, cut in serial cross-sections of 10 μ thickness, and stained in iron-lac-hematoxylin. The series includes 373 sections from the frontal end of the embryonic shield to the caudal end of the allantois. The neural groove is closed through the greater part of its extent, except for a short distance in its most caudal portion. At the level of the seventh to the tenth somites the intermediate cell mass is seen to be differentiating from the dorsomedial wall of the coelomic cavity. This cell mass is sometimes closely connected with the coelomic wall; sometimes it is of a more nearly round shape and related to the coelom by a narrow band of cells, so that the whole structure has the appearance of a club (fig. 3, left side). In certain sections the intermediate cell mass is quite independent of the coelomic mesoderm. In certain sections the pleuroperitoneal wall reveals a shallow groove at the place of its connection with the intermediate cell mass. Occasionally this groove continues farther into the latter cell mass as a narrow canal (fig. 3, right side). Such grooves and canals communicating directly with the coelomic cavity may be considered as the homologue of the rudimentary pronephric tubules.

At the level of the eleventh somite the intermediate mesoderm is often free from a connection with the pleuroperitoneal wall; sometimes related to it only by a narrow cell bridge. Usually no connection with the somites is observable.

At the level of the twelfth somite a more nearly round cell mass becomes delimited from the dorsal portion of the intermediate mesoderm. This round cell mass is considered as the anlage of the mesonephric duct. It is entirely separated from the intermediate mesoderm at the level of the caudal end of the twelfth somite; in certain sections it is provided with a small lumen. The intermediate cell mass is here usually not connected either with the somitic or with the coelomic mesoderm, though its ventrolateral angle lies very close to the latter. At the level of the thirteenth somite the intermediate cell mass is connected with the coelomic wall and sometimes also with the somite. The mesonephric duct becomes smaller and lies now nearer to the ectoderm, which forms an outwardly concave fold at the corresponding place. The lumen of the duct is very clear. At the level of the caudal portion

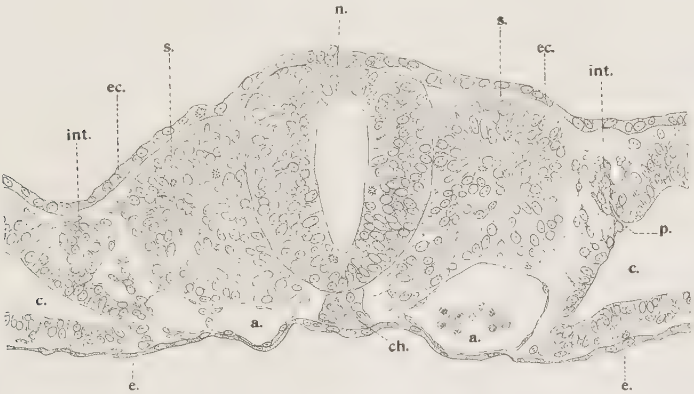

Fig. 3 Cross-section through the level of the ninth somite of a rabbit embryo of fourteen somites. Camera-lucida drawing. × 182. *a.*, aorta; *c.*, coelom; *ch.*, notochord; *e.*, entoderm; *ec.*, ectoderm; *int.*, intermediate cell mass; *n.*, neural tube; *p.*, homologue of a rudimentary pronephric tubule; *s.*, somite.

of the thirteenth somite the intermediate cell mass is connected with both the coelomic and the somitic mesoderm. At the level of the fourteenth somite the intermediate cell mass appears in the form of a band of cells stretched out between the somite and the coelomic wall. The mesonephric duct becomes smaller and loses its lumen. However, it continues a little farther caudad than the level of the fourteenth somite, disappearing sooner on one side than on the other. The caudal end of the duct lies quite close to the ectoderm, as in the above-described guinea-pig embryo of fourteen somites. However, a delimitation from the ectoderm is made very easily in the rabbit embryo. In the rabbit embryo the basement membrane (membrana prima) of the ectoderm is often interrupted at places where the mesonephric duct lies near the ectoderm. It appears that sometimes, instead of lying close to the latter, the membrane withdraws from it slightly at these places and lies at the side of the mesonephric duct adjacent to the ectoderm or even seems to be fused with the mesonephric duct, while a small space may be present between the duct and the ectodermal cells. Graf Spee described a basement membrane on that side of the mesonephric duct which faces the intermediate cell mass. Such a condition is not observable in our material.

In the rabbit embryo of fourteen somites, as in the guinea-pig embryo of the same number of somites, the mesonephric duct is recognizable at the level of the twelfth to the fourteenth somites. In both embryos the duct is fused in its frontal portion with the intermediate cell mass and differentiates as a bud of the latter. Caudally, the mesonephric duct separates from the intermediate cell mass; it becomes smaller and lies very close to the ectoderm. However, in both embryos the elements of the mesonephric duct are always differentiable from those of the ectoderm. In the rabbit embryo, also, rudimentary homologues of the pronephric tubules may be identified at levels frontal to the mesonephric duct. The intermediate cell mass in the rabbit embryo is continuous in its frontal part with the coelomic wall, but usually free from any connection with the somites.

I wish to express my appreciation to Professor Huber for suggesting this study, for the privilege of using his embryological material, and for his direction of the work.

## SUMMARY

The mesonephric duct differentiates in guinea-pig and rabbit from the intermediate cell mass of the mesoderm. It makes its appearance as a solid rod of cells, which at its caudal end lies very close to the ectoderm. Especially in its caudal portion, it is often continuous with the somitic mesoderm.

The material studied by us does not supply any evidence for the assumption of a histogenetic participation of the ectoderm in the development of the primary excretory duct in mammals.

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# A UNIQUE MODEL OF THE ENTIRE PERITONEUM

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TWO PLATES (TWO FIGURES)

The peritoneum is difficult for the student to understand and difficult for the teacher to explain or demonstrate; therefore, I have tried for about twenty years to teach an imaginary peritoneum with all the viscera removed. About a year ago, Medicina in Chicago offered to make the model that I had imagined for so many years, and they finished the model this year under my direction and instructions.

The model is made to represent nothing but the peritoneum, although the body wall is retained on each side and at the base to support the model. The structure of the model is tough and strong, made by a special process, and although the model is thin it resists considerable strains.

Two views are given, one from the front and the other from the rear. The front view is the conventional one, but the rear view is unusual. The front shows the ventral parietal peritoneum removed, a large part of the great omentum cut away with a section of the transverse colon; and the jejunum and ileum, as well as the descending and sigmoid parts of the colon, cut off, so that a better view may be obtained of the remainder. A hole is cut in the lesser omentum, and through this may be seen a string which passes out through the epiploic foramen, and at the other end goes below the stomach and above the cut mesentery of the transverse colon, in front of the cut end of the duodenum. The diaphragm is slightly elevated to show the liver, and the liver is also slightly elevated to show the structures underlying it; otherwise the model is fairly normal.

The four layers of peritoneum that make up the great omentum as it hangs in the abdomen are distinct, and the way the ventral layer comes from above the stomach and passes as the dorsal layer beneath the mesentery of the transverse colon is clear, as also is the way the layer beneath this passes from behind the stomach and returns to form the upper layer of the mesentery of the transverse colon.

The upper end of the spleen is clearly shown, and the cut edge of the mesentery of the sigmoid, as well as a part of the space where the lower end of the transverse colon lay. A great deal more may be seen than is in the illustrations because one object obscures another.

The upper part of the stomach is in view, and the lower end of the caecum with the appendix coming from behind it. The contour of the duodenum in part may be followed and the eminence of the pancreas discerned.

The diagonal arrangement of the viscera in layers may be understood by following the lower border of the liver, the greater curvature of the stomach, the transverse colon, the mesentery of the small intestine, and the mesentery of the sigmoid and lower end of the descending colon, as they lie from above on the left downward to the right.

The depressions of the peritoneum when in the recumbent posture may be seen beside the vertebral column, with a separation of each depression into two fossae, by the ascending colon on the right and the descending colon on the left. The central prominence divides below around the brim of the pelvis, as the psoas muscles and the iliac arteries and veins. This causes obstructions to the fossae at the pelvis, but the lateral fossae continue upward to the diaphragm without obstruction, around the spleen on the left and the liver on the right, thus reaching the peritoneum of the diaphragm. The medial fossae are blocked above by the mesentery of the transverse colon. Fluid in the peritoneal space may overflow into the pelvis from the medial fossae, and it may reach the region of the diaphragm through the lateral fossae, when the person is in the recumbent posture.

This is important in certain conditions, such as postoperative treatment, because fluids are absorbed more rapidly through the peritoneum in the region of the diaphragm than through the peritoneum in the pelvis, and fluids absorbed rapidly after operations increase shock. The prominences made by the vertebral column, the psoas muscles, and iliac arteries and veins may be seen in the model, as well as the depressions and fossae.

There is nothing especially new about the front view of the model, although it is convenient and illuminating. The view from the rear is unique and instructive.

Starting above, a cut is shown in the peritoneum below the diaphragm through which the upper surface of the liver may be seen. To the left of this is the slit occupied by the falciform ligament of the liver. The slit passes diagonally downward and toward the right to the cavity from which the liver has been removed. The opening of this cavity represents the bare surface of the liver where the peritoneum does not extend. The cavity curves to a blunt point on the right and a sharp point on the left, and between these two is a slit, below which is the vestibule of the omental bursa that passes up behind the liver. To the right and below the vestibule is the impression of the inferior vena cava, and to the left of the vestibule is the slit made by the esophagus before it enters the stomach. Following from this slit farther to the left is the irregular opening which represents the hilus of the spleen, the left end of which becomes continuous with the cavity of the splenic flexure of the colon. This may be traced downward to where it becomes the narrow slit which represents the mesentery of the sigmoid that extends upward and to the right to end in the impression left by the rectum about the middle of the lower end of the model.

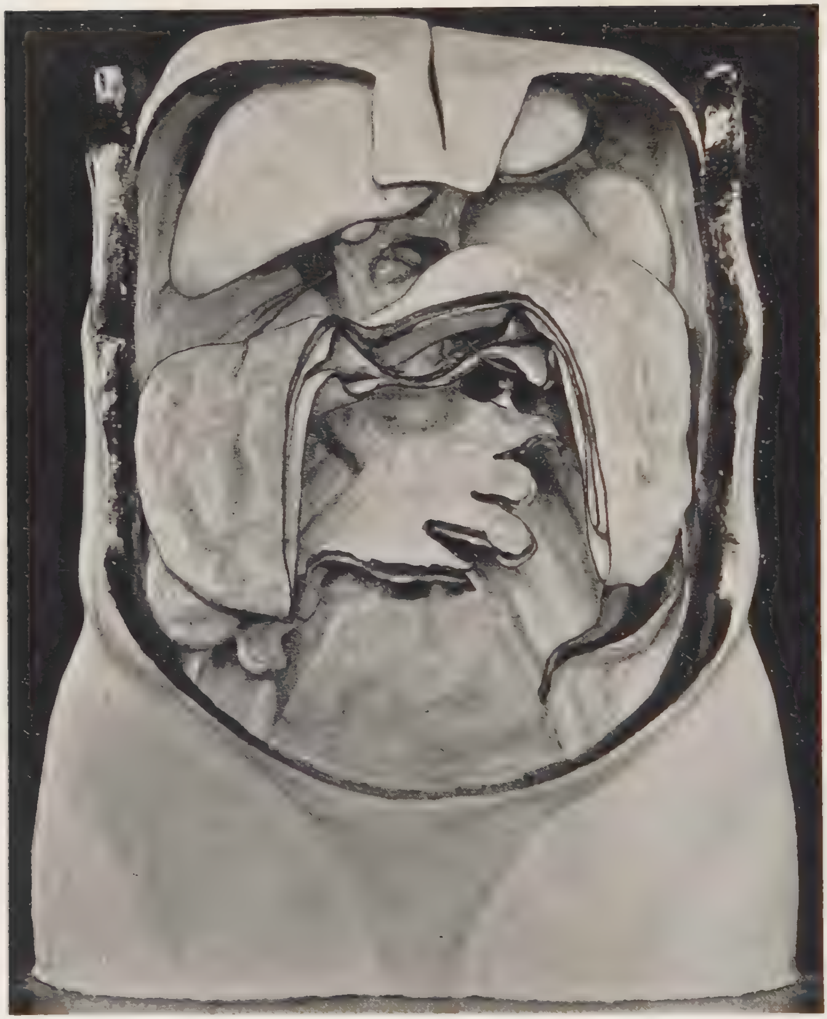
Going back to the slit which represents the hilus of the spleen immediately above the splenic flexure of the colon, the narrow transverse slit represents the mesentery of the transverse colon, which may be followed to the right until it broadens into the hepatic flexure of the colon, which continues down as the space for the ascending colon and the caecum. To the left of this impression is the slit for the mesentery of the small intestine which may be followed diagonally upward to the left to terminate in the hole in white which represents the opening of the duodenum into the jejunum. Curving from this hole downward and to the right, then upward and farther to the right, is the impression of the duodenum which terminates in the pylorus of the stomach. The impression of the duodenum crosses the slit of the mesentery of the small intestine and the slit of the mesentery of the transverse colon.

The impression of the pancreas may be seen in the curve of the duodenal impression where the head of the pancreas lies, upward to the vestibule of the omental bursa, thence to the left of the hilus of the spleen. The impression of the tail of the pancreas is seen above the left end of the slit of the mesentery of the transverse colon. Above the tail of the pancreas and below the hilus of the spleen is a triangular area made by a part of the left kidney; and below the tail of the pancreas and between the beginning of the duodenum and the hepatic flexure of the colon is a larger area also made by the left kidney. The pancreas and the mesentery of the transverse colon here cross ventral to the left kidney. To the right of the impression made by the pancreas and to the right of the impression made by the duodenum is the impression of a part of the right kidney. The pancreas and the duodenum are here in contact with the right kidney, and the right kidney here lies ventral to the transverse mesocolon and is in contact with the hepatic flexure of the colon.

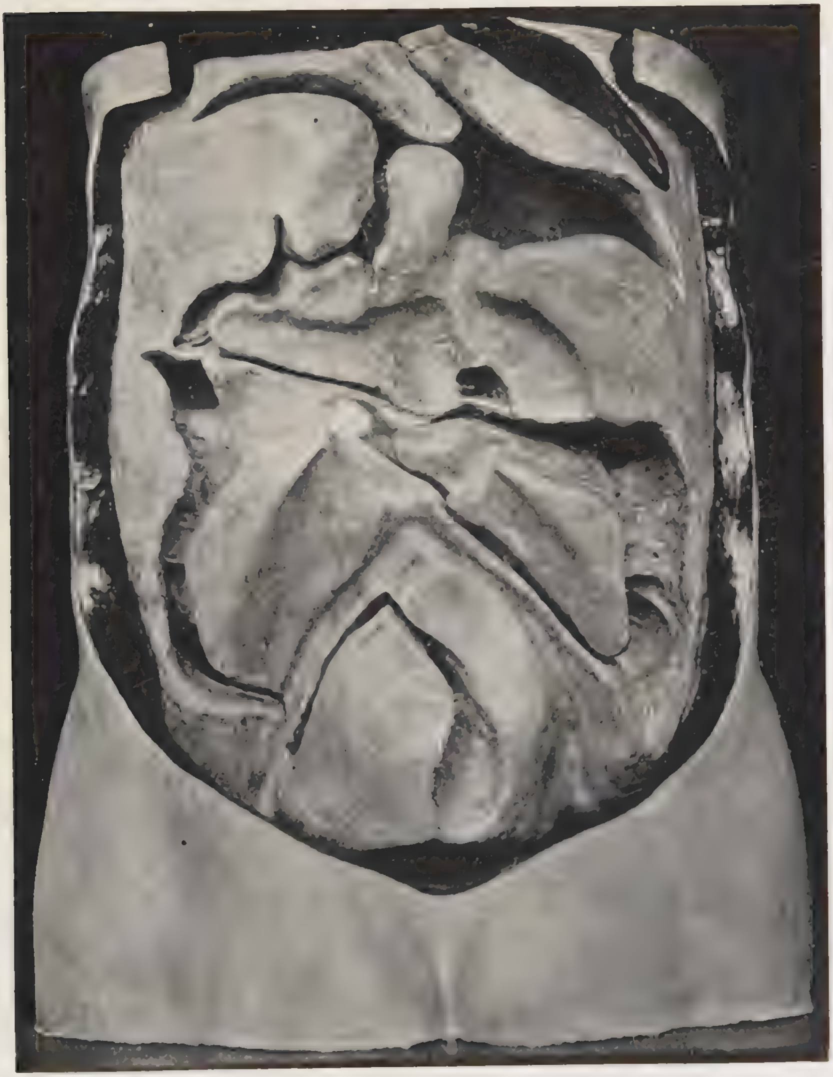
Below the impression of the duodenum is the impression of the bifurcation of the aorta and the iliac arteries. Lateral to these are the larger impressions of the psoas muscles. Immediately below the bifurcation of the aorta is a short impression of the left iliac vein just below where the right iliac artery crosses it. Between the iliac arteries and above the impression of the rectum is a large shallow depression for the promontory of the sacrum and lower lumbar vertebrae.

When the model is looked at from above, the depression seen in the middle of the back represents the vertebral column, and the elevations on each side represent the fossae that are seen in front. The central depression bifurcates below, where the deep furrows made by the psoas muscles and iliac arteries may be seen.

The diagonal attachment of the viscera may be seen where the left triangular ligament of the liver is higher than the right, the cardiac orifice of the stomach is higher than the pyloric orifice, the splenic flexure of the colon is higher than the hepatic, and the duodenal end of the small intestine is higher than the iliac end. The diagonal sigmoid is not shown, as its mesentery is somewhat S-shaped.



Front view



Rear view

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